JOURNAL OF AGRICULTURAL RESEARCH
DEPARTMENT OF AGRICULTURE

VOL. II WASHINGTON, D. C., AUGUST 15, 1914 NO. 5

FRUIT-ROT, LEAF-SPOT, AND STEM-BLIGHT OF THE EGGPLANT CAUSED BY PHOMOPSIS VEXANS

By L. L. HARTER,
Pathologist, Cotton and Truck Disease and Sugar-Plant Investigations, Bureau of Plant Industry

INTRODUCTION

During the summer of 1912 when searching for eggplants (*Solanum melongena*) affected with stem-rot, supposedly caused by a Fusarium, Mr. G. F. Miles, then pathologist in the Office of Cotton and Truck Disease and Sugar-Plant Investigations, sent the writer some full-grown plants from New Jersey which had the appearance of wilt. The epidermis of the stem for 3 or 4 inches above the soil line was injured and the fibro-vascular bundles blackened. Cultures from the blackened bundles yielded in a few days not a Fusarium but an organism which, because it was isolated from the stem and otherwise agreed with Halsted's description, was regarded as *Phoma solani*.

A disease of the leaf and fruit of the eggplant, commonly attributed to *Phyllosticta hortorum* Speg. has been known to plant pathologists as very prevalent in this country and certain parts of Europe. However, after some study of the organism, Smith (1905, p. 10)[1] concluded that the pycnospores were 2-celled, and proposed the name "*Ascochyta hortorum* (Speg.)." Judging from reports which have appeared since that time, pathologists in general have not accepted the suggested change, but have continued to refer to the organism as *Phyllosticta hortorum* Speg.

The writer regarded the organism on the leaf and fruit as a Phyllosticta and believed that *Phyllosticta hortorum*, *Phoma solani*, and *Ascochyta hortorum* were one and the same fungus. Cross-inoculations were started with *Phoma solani* Hals. and with an organism isolated in 1912 from diseased fruit of eggplants by Mr. A. G. Johnson,[2] of the University of

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 338.

[2] The writer is also indebted to Mr. A. G. Johnson for the use of embedded material and to Dr. I. E. Melhus for the loan of preserved specimens.

Wisconsin, who very kindly gave the writer a culture for comparative studies. The results of inoculation experiments showed that both *Phoma solani* and *Phyllosticta hortorum* are able to produce a fruit-rot and a stem-blight of eggplants. When this fact was determined, morphological studies were made of the two fungi. As a result of this study, the fungi were found identical, and, furthermore, it was concluded that the genus to which they belonged was neither Phoma, Phyllosticta, nor Ascochyta.

DESCRIPTION OF THE DISEASE

The fungus causing damping-off, or seedling stem-blight, of young eggplants and seedlings was attributed by Halsted (1892, p. 277) to the fungus *Phoma solani*. The stems of seedlings or very young plants attacked by the fungus are girdled for an inch or more above the soil line. The plants soon topple over and die (Pl. XXVI). The part of the stem girdled by the fungus has a smaller diameter than the healthy portion above. This is due partly to the falling away or drying up of the diseased tissue and partly to the arrest in the growth of the stem where the fungus is present. Although pycnidia are usually formed abundantly on the diseased stems of young plants (Pl. XXVII), they develop sparingly or not at all on older ones. They may be developed, however, on old plants by placing the diseased stem for a few days in a moist chamber.

On the leaves (Pl. XXVIII) the fungus causes in the earlier stages brown, dead, round, oval, or oblong spots which become more irregular in shape and jagged in outline with age. The irregularly shaped spots, varying from 2 mm. to 2 or 3 cm. in diameter, are more prevalent on or near the margin of the leaf, or along the midrib or larger veins. These spots consist of a light or grayish inner zone surrounded by a darker, almost black, margin of perhaps ¼ to ½ mm. in width. They are usually not concentrically enlarged, although their appearance sometimes is such. Two or more spots may unite, forming large blotches, and upon the midrib, the petiole, or upon the large veins of the leaf the fungus may produce lesions or abrasions in which pycnidia are formed.

When the fruit is attacked by this organism, it becomes at first soft and mushy, but later mummified and black (Pl. XXIX, fig. 1). A pure culture can usually be secured by planting bits of the inner tissue (Pl. XXX) in plates of agar. Pycnidia may form at first in rather definite spots, but in most cases they will finally cover the whole surface of the fruit. It is believed that young fruit is most subject to attack, although fruit in all stages of growth has been found to be diseased.

The pycnidia, often with a well-defined beak, are at first buried, but later break through the epidermis and appear as brown or black specks extending a little above the surface. On the fruit the pycnidia become visible to the naked eye and are considerably larger than those on the leaves. They stand close together on both the fruit and leaves, separate

in most cases, though occasionally they unite. The pycnidia on the stem are about equal in size to those on the leaf, but they are fewer, except on the stem of very young seedlings. (See Pl. XXVII.) On the leaves the pycnidia vary from 60 to 200μ in diameter, while on the fruit they measure 120 to 350μ.

Stylospores, the filiform, hooked-shaped bodies, were found abundantly in the pycnidia on the fruit and stems of many plants inoculated in a greenhouse of the Department of Agriculture and on the Potomac Flats, near Washington, D. C. If they were not present on the stem when the plants were lifted, they would frequently develop if kept a few days in a moist chamber. They were also occasionally found in cultures on corn meal.

INOCULATION EXPERIMENTS

The pathogenicity and relationship of the fungi isolated from the fruit and from the stem of eggplants are shown by the results of inoculation experiments recorded in Table I.

In several experiments the plants were covered for a day or two before spraying and for 24 to 48 hours after with paper-wrapped bell jars or paper-wrapped glass infection cases. This method, however, did not appear to influence the results, since plants which were not covered before or after spraying were likewise infected. In fact, mature plants on the Potomac Flats, near Washington, D. C., were sprayed at 11 a. m. on a very warm, partly cloudy day and left uncovered, and numerous infections of fruit and leaves took place. A few infections were found on the check plants. It is believed, however, that they came from the sprayed plants, since no eggplants were grown within a mile of the experiment, so far as could be determined, and since the check plants nearest those sprayed showed the worst spots. In every experiment with *Lycopersicon esculentum*, *Datura tatula*, and *Capsicum annuum* the plants were covered for 48 hours after spraying.

In all, 27 sets of inoculation experiments have been carried out. Sixty-one eggplants in 7 sets were inoculated by inserting spores and hyphæ of the different organisms into the lower part of the stem, and 59, or nearly 97 per cent, of these plants became infected. Fifty eggplants in 8 sets were sprayed with spores of the different fungi suspended in water, and 47, or 94 per cent, were to some degree infected. Two pots containing many seedlings of eggplants each were sprayed with spores in suspension, and practically all succumbed to the disease. Six half-grown eggplant fruits were sprayed in 2 sets with spores in suspension, and 5 rotted from the effects of the organism. Ten sweet-potato plants (*Ipomœa batatas*) were inoculated at the base of the stem, but none became diseased. Six large tomato plants in 2 sets and 14 small plants in 2 sets were sprayed with a suspension of spores, but none became diseased. Twenty pepper plants (*Capsicum annuum*) in 2 sets and 10 plants of *Datura tatula* in 1 set were sprayed with a suspension of spores, but none were infected.

TABLE I.—*Results of inoculation experiments on eggplant with Phoma solani and Phyllosticta hortorum from various sources*

Organism No.	Organism.	Name of host.	Age of host.	Place of inoculation.	Method of inoculation.	Number inoculated.	Number infected	Number of checks.	Number of checks infected.
a 104	Phoma solani	Solanum melongena	Half-grown	Potomac Flats	Foliage sprayed with spores suspended in water.	6	6	6	(*g*)
b 113	Phyllosticta hortorum	do	do	do	do	6	6	6	(*g*)
104	Phoma solani	do	Mature (fruiting)	Garden near greenhouse	do	2	2	0	.....
113	Phyllosticta hortorum	do	do	do	do	2	2	0	.....
104	Phoma solani	do	Medium young	Greenhouse	do	8	5	6	0
113	Phyllosticta hortorum	do	Half-grown	do	do	10	10	5	0
104	Phoma solani	do	Mature	do	do	10	10	5	0
113	Phyllosticta hortorum	do	Seedlings	do	do	Many.	Many	Many.	0
113	do	Lycopersicon esculentum	Mature (fruiting)	do	do	3	0	Many.	0
104	Phoma solani	Capsicum annuum	do	do	do	6	0	0	.....
104	do	Lycopersicon esculentum	Half-grown	do	do	8	0	0	.....
104	do	Datura tatula	Medium young	do	do	10	0	0	.....
c 116	Phyllosticta hortorum	Solanum melongena	Mature	do	do	6	6	0	.....
d 109	Phoma solani	do	Seedlings	do	do	Many	Many	Many.	0
115	Phyllosticta hortorum	Lycopersicon esculentum	Mature (fruiting)	do	do	3	0	Many.	0
116	do	do	Young	do	do	6	0	Many.	0
116	do	Capsicum annuum	do	do	do	14	0	Many.	0
104	Phoma solani	Solanum melongena (fruit).	Half-grown	Laboratory	do	3	3	0	.....
113	Phyllosticta hortorum	do	do	do	do	3	2	0	.....
104	Phoma solani	Solanum melongena	Young	Greenhouse	Spores and hyphæ inserted into the lower part of stem.	10	10	6	0
104	do	do	Mature	do	do	7	6	5	0
104	do	do	Young	do	do	10	10	5	0
104	do	Ipomœa batatas	do	do	do	10	0	10	0
113	Phyllosticta hortorum	Solanum melongena	do	do	do	10	9	0	.....
109	Phoma solani	do	do	do	do	8	8	6	0
e 112	do	do	Half-grown	do	do	10	10	5	0
f 115	Phyllosticta hortorum	do	Young	do	do	6	6	6	0

a Isolated from the dead tissue of a diseased stem of eggplant sent the writer from New Jersey by Geo. F. Miles.
b Isolated from the fruit of eggplants by A. G. Johnson, Madison, Wis.
c Recovered from the stem of eggplants inoculated in the greenhouse with organism No. 113. Stylospores were present on the plant from which the isolation was made.
d Recovered from a plant inoculated with organism No. 104.
e Isolated from the stem of a plant inoculated with organism No. 104, on which stylospores were found.
f Recovered from the leaves of eggplants sprayed with spores of organism No. 113.
g All very slightly.

It is evident from a detailed study of the inoculation experiments that the fungus *Phoma solani* isolated from the stem of eggplant will also infect the fruit and leaves of the eggplant and that the fungus isolated from the fruit (*Phyllosticta hortorum*) will infect the stem and leaves. Both of these organisms will cause a rapid damping-off of eggplant seedlings. Judging from the results of the experiments here performed, both fungi are parasitic on *Solanum melongena* at any age, but not on *Ipomoea batatas, Lycopersicon esculentum, Capsicum annuum,* and *Datura tatula.* A careful study of infected plants show that the injuries produced by the two organisms are indistinguishable and that the evidence of infection after inoculation is manifested in about the same length of time.

No attempt has been made to recover the organisms from all infected plants. It has, however, been isolated from many infected leaves, fruit, and stems. Many plants have been inoculated with cultures of the organism recovered from previously inoculated plants, and the organism has been recovered a second time from some of these infected plants. In fact, the four cardinal requirements, known as Koch's rules, have been fulfilled in a number of instances as proof of the pathogenicity of the organisms used in the inoculation experiments. In view of results gained from inoculation experiments, it is evident that the two fungi, known as *Phoma solani* and *Phyllosticta hortorum*, are identical. They are also identical morphologically.

TAXONOMY OF THE FUNGUS

Spegazzini (1881, p. 67) described a fungus occurring on the leaves of *Solanum melongena* as *Phyllosticta hortorum*, the pycnidia of which measured 80 to 90μ in diameter and the pycnospores 4 to 6μ long and 2 to 2.5μ wide. Halsted (1892, p. 279) reported the same fungus on the leaves and fruit of *Solanum melongena* in New Jersey, and at the same time he also reported (1892, p. 277) damping-off or seedling stem-blight of eggplant, which he described as *Phoma solani.* Smith (1904) published a short note to the effect that he had found *Ascochyta lycopersici* on the leaves and fruit of *Solanum melongena.* He says "the fungus differs from *Phyllosticta hortorum* Speg., both in size and septation of spores and in character of leaf spot." He further says that "a careful comparison with Halsted's material showed the two to be distinct"; also that "the spores of *Phyllosticta hortorum* Speg., in material collected by Halsted agree in size with those given by Saccardo, 4–6×2–2.5μ, while those in this Ascochyta are nearly twice that size, 6–12×3.5–4μ." Smith, with the fungus he had, was able to infect *Solanum melongena, Lycopersicon esculentum, Solanum carolinense,* and *Datura tatula.* The following year he (Smith, 1905, p. 10-14) seems to have thought that the organism he had under observation the year before was *Phyllosticta hortorum* Speg., which "manifested slightly different characteristics from that of the previous year." According to his obser-

vations, "the fungus produced more numerous, as well as more prominent, fruit bodies. The spores were somewhat smaller and the spots lighter colored. In these respects the disease resembled more closely material collected by Halsted and labeled *Phyllosticta hortorum*." It appears evident from Smith's second article that he regarded *Phyllosticta hortorum* identical with *Ascochyta lycopersici*. If his conclusions are accepted, *Phyllosticta hortorum*, having the priority, would be *Ascochyta hortorum* (Speg.) Smith.

Voglino (1907) in Italy worked with a fungus on eggplants which he thought to be the same as that described by Spegazzini as *Phyllosticta hortorum*. By a series of inoculation experiments with spores of an Ascochyta found on *Solanum melongena* he was able to induce infection on *Physalis alkekengi*, *Solanum nigrum*, *S. dulcamara*, *Lycopersicon esculentum*, *Datura metel*, and *Atropa belladonna*. Voglino agrees with Smith that the fungus previously described by Spegazzini as *Phyllosticta hortorum* is

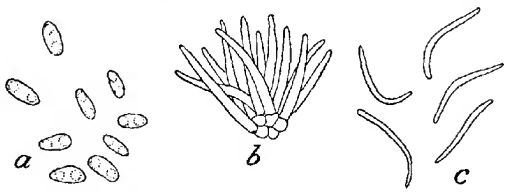


FIG. 1.—Some microscopic characters of the fungus *Phomopsis texana*: *a*, Pycnospores; *b*, conidiophores; *c*, stylospores.

an Ascochyta—*Ascochyta hortorum* (Speg.) Smith—and devotes considerable space to a discussion of his reason. To this species must be referred, he says, "*Ascochyta lycopersici* Brun. (*A. socia* Passerini), *A. solanicola* Oudemans, *A. atropae* Bresadola, *A. alkekengi* Massalongo (*A. pedemontana* Ferraris), *A. physalicola* Oud. and perhaps *A. prinzalensis* B. and K."

The writer has made a careful morphological study of the fungus identified as *Phyllosticta hortorum* and collected from the following places: Glen Cove, N. Y. (collected by Pries and identified by Whetzel); Starkville, Miss. (Tracy); Lincoln, Nebr. (Heald); New Brunswick, N. J. (collected by Halsted and identified by Seymour and Earle). The pycnidia in each case were typical of Phomopsis. Specimens collected by Melhus at Madison, Wis., in 1912, already referred to, were examined and found to be a Phomopsis. The pycnidia on specimens from the different localities were more or less beaked, flattened, or irregular in form. They were covered with a thick, black wall (Pl. XXIX, fig. 2) which becomes thinner and less noticeable at the base. The conidia (fig. 1, *a*) were 1-celled, with mostly two, sometimes three oil droplets [1]; the coni-

[1] In rare cases spores might be found, the contents of which appeared divided, but if they were treated with a solution of salicylic acid, the division would frequently disappear, showing that the spores are continuous. The apparent division is merely caused by two vacuoles or oil globules which meet at the center of the conidia.

diophores stout and awl-shaped (fig. 1, *b*). Stylospores (fig. 1, *c*) were found on herbarium specimens from Ithaca, N. Y., on specimens from Wisconsin loaned by Dr. Melhus, and on specimens collected by the writer in New Jersey, on the stem and fruit of inoculated plants, and occasionally in artificial cultures. These characters just mentioned are according to Diedicke (1911) typical of the genus Phomopsis. The spores vary in size from 5 to 8 by 2 to 2.5μ. The morphological characters of the pycnidia on specimens from the various States seem to agree with each other and with specimens produced by the writer as a result of inoculation. A careful study of the fungus with which the writer has worked shows that *Phoma solani* is identical with the fungus causing the fruit-rot and leaf-spot of eggplants and that it belongs to the genus Phomopsis. Attempts to infect *Lycopersicon esculentum*, *Capsicum annuum*, and *Datura tatula* have been unsuccessful.

Owing to the fact that Phomopsis was widely distributed in the United States on *Solanum melongena*, doubt finally arose in the mind of the writer whether *Phyllosticta hortorum* Speg. occurs in this country. Typical specimens of the disease were therefore sent to Spegazzini for examination. After comparing them with his own type specimens, he said that the fungus was not *Phyllosticta hortorum* and pointed out the characteristic differences in the spots, pycnidia, and spores of the two fungi. If, therefore, any value is to be given to a comparison made by an author with his own type specimens, it is safe to conclude that *Phyllosticta hortorum* has not yet been found in this country. How, then, can Smith's results and those of Voglino be explained? The writer is of the opinion that Smith had an Ascochyta on the eggplant, but at the same time another fungus, the so-called *Phyllosticta hortorum*. The writer has examined specimens of Ascochyta on eggplant collected by Whetzel in New York and identified by Jensen as *Ascochyta lycopersici*. The spores are 2-celled and agree in size with spores of *Ascochyta lycopersici*, 6 to 10 by 2.5μ. It is probable that in 1904 Smith had this Ascochyta under observation and in 1905 observed the fungus generally known as *Phyllosticta hortorum*, since he says that the fungus in 1905 produced more numerous as well as more prominent fruit bodies. Such a distinction is certainly true of these two genera as they appear on eggplant. *Ascochyta lycopersici* occurs on both eggplant and tomato, and Smith was able to cross-inoculate these two hosts. The writer, on the other hand, was unable to infect *Lycopersicon esculentum*, *Capsicum annuum*, or *Datura tatula* with the organism he studied. Voglino (1913, p. 213–218) calls attention to a disease of the eggplant, tomato, and pepper which, as a result of cross inoculations, he believes to be caused by *Ascochyta hortorum*. The spores, however, are considerably larger than those of *Phyllosticta hortorum*, 10 by 3μ, and it is likely that he also had *Ascochyta lycopersici*.

The fungus with which the writer has worked is assigned to the genus Phomopsis because it possesses the following characteristics of that genus:

(1) Stylospores; (2) irregularly shaped or flattened pycnidia, with a well-developed beak; (3) long, stout, and awl-shaped conidiophores; (4) thick, black wall at the top of the pycnidia, becoming less noticeable at the base. It forms a stroma in culture and beaks 1 mm. or more in length.

The combination *Phomopsis vexans* is proposed as the name for the fungus. In 1892 Halsted assigned the name *Phoma solani* to the organism causing the damping-off of the eggplant, apparently not being aware that it was preoccupied by Cooke and Harkness for a fungus on another host. In view of that fact, Saccardo and Sydow substituted *Phoma vexans* for *Phoma solani* Hals.

Phomopsis vexans (Sacc. and Syd.), n. comb.

Phoma solani Hals., 1892, *in* N. J. Agr. Expt. Sta., 12th Ann. Rpt., 1891, p. 277. nom. nud. Sacc., 1895, Syll. Fung., v. 11, pars 3, p. 490. Not Cooke and Hark., 1884, *in* Grevillea, v. 13, p. 16.

Phoma vexans Sacc. and Syd., 1899, *in* Sacc. Syll. Fung., v. 14, p. 889.

Ascochyta hortorum (Speg.) C. O. Sm., 1905, *in* Del. Agr. Expt. Sta. Bul. 70, p. 10–14. err. det. Not *Phyllosticta hortorum* Speg.

On the foliage and stems pycnidia loosely gregarious in more or less definite spots, on fruit compact, at first buried, later erumpent, black without, beaked, flattened or irregular in shape, on leaves and stems 60 to 200μ broad, on fruit 120 to 350μ broad; pycnospores subcylindrical, somewhat acute, 5 to 8 by 2 to 2.8μ, continuous, hyaline, 2-guttulate, rarely 3; basidia simple, short, straight or slightly curved, hyaline, continuous; stylospores filiform, curved, rarely straight, 13 to 28μ long.

Hab. on leaves, stem, and fruit of *Solanum melongena*. Type specimens deposited in the herbarium of the pathological collections of the Bureau of Plant Industry, Department of Agriculture, Washington, D. C.

LITERATURE CITED

DIEDICKE, HERMANN.

1911. Die Gattung Phomopsis. Ann. Mycol., v. 9, no. 1, p. 8–35, pl. 3.

HALSTED, B. D.

1892. Some fungous diseases of the egg-plant. N. J. Agr. Expt. Sta., 12th Ann. Rpt. 1891, p. 277–283, fig. 16–22.

SMITH, C. O.

1904. A new egg plant fungus. Jour. Mycol., v. 10, p. 98–99, 6 fig. May.

1905. Study of the diseases of some truck crops of Delaware. Del. Agr. Expt. Sta. Bul. 70, 16 p., 6 fig., 2 pl.

SPEGAZZINI, CARLOS.

1881. Nova addenda ad mycologiam venetam. Atti, Soc. Crittog. Ital., ann. 24, s. 2, v. 3, disp. 1, p. 42–71.

VOGLINO, PIETRO.

1907. Intorno ad un parassita dannoso al *Solanum Melongena*. Malpighia, ann. 21, fasc. 7/8, p. 353–363, pl. 4.

1913. I funghi più dannosi alle piante osservati nella provincia di Torino e regioni vicine nel 1911. Ann. R. Accad. Agr. Torino, v. 55, 1912, p. 199–227.

PLATE XXVI

Damping-off of seedling eggplants which had been sprayed with organism No. 113 (*Phyllosticta hortorum*). All the plants were finally killed by the fungus.

PLATE XXVII

Seedling eggplants from the pot shown in Plate XXVI (enlarged), showing the presence of pycnidia and the effect of the fungus on the stem.

PLATE XXVIII

An eggplant leaf sprayed with organism No. 104 (*Phoma solani*), showing the characteristic spots and pycnidia formed therein.

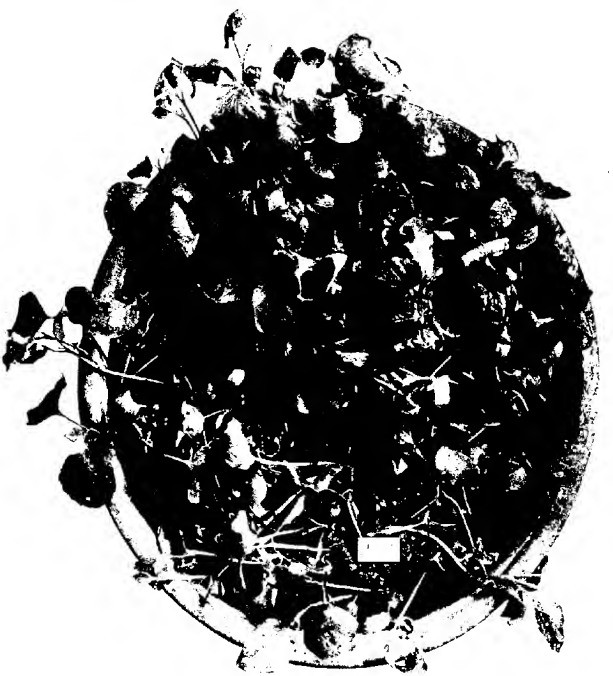
PLATE XXIX

Fig. 1.—An eggplant fruit produced by a plant grown on the Potomac Flats and mummified as a result of spraying with organism No. 104 (*Phoma solani*). Note the pycnidia on the surface.

Fig. 2.—A photomicrograph of a cross section through pycnidia formed on the calyx of an eggplant. Note the thick black wall covering the pycnidia.

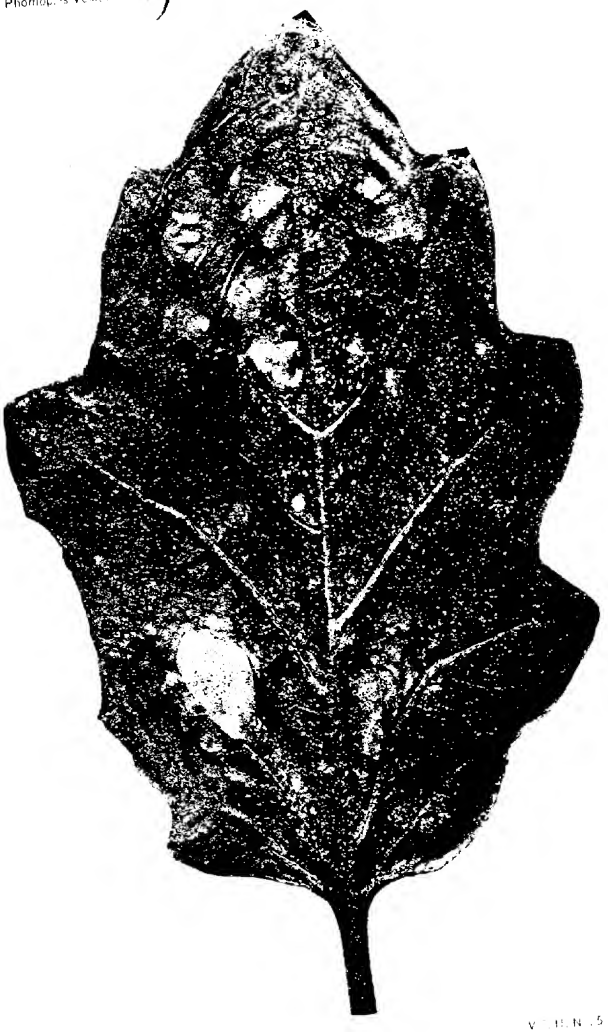
PLATE XXX

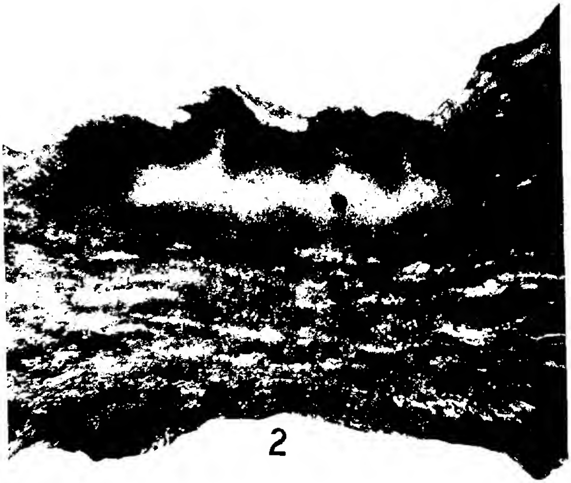
A section through the fruit of an eggplant which had been sprayed in a moist chamber with organism No. 113 (*Phyllosticta hortorum*). The fungus had entered and softened the fruit to the depth shown by the darkened portion of the photograph and was recovered in pure culture from the rotted interior. Other fruits similarly treated and left longer in the moist chamber were completely rotted.



Physalis Viscosa var. Elegans

Phomopsis Vexans (Sacc. & Syd.) Harter





2

HEAD SMUT OF SORGHUM AND MAIZE

By ALDEN A. POTTER,
Assistant Pathologist, Office of Cereal Investigations,
Bureau of Plant Industry

GENERAL CHARACTERISTICS OF THE DISEASE

DISTRIBUTION

In the agriculture of western Kansas and Texas and similar parts of the Great Plains area various sorghum varieties have recently attained considerable importance as a dry-land crop in the farming operations which are developing in the sections formerly devoted to cattle ranges. This fact, together with the importance of broom corn in some sections, has led to an investigation of the diseases of the sorghum crop by the Office of Cereal Investigations of the Bureau of Plant Industry.

The study of the head smut has an added importance from the fact that it occurs on maize (Indian corn) and has been reported by McAlpine (1910, p. 290)[1] as serious on that crop in Australia, and by Evans (1911) and Mundy (1910, p. 1) in South Africa (Pl. XXXI). It has been found on maize in some abundance in this country (Norton, 1895; Hitchcock and Norton, 1896, p. 198), although the writer, in rather extensive observations, has never seen such a case; nor has it been recently reported.

The parasite is widely distributed in sorghum-growing regions throughout the world, and in some sections, chiefly tropical or subtropical, it is very destructive. Munerati (1910, p. 718) has found it abundant on *Sorghum halepensis*, and it has also been reported from Italy by Passerini (1877, p. 236), Mottareale (1903, p. 3), and Cugini (1891, p. 83); from India by Cooke (1876, p. 115) and Barber (1904); from Egypt by Kühn (1878, p. 10); from German East Africa by Busse (1904, p. 378); and from Japan by Hori (1907, p. 163). According to Hennings (1896, p. 119), it occurs in North and East Africa, Madagascar, and East India, as well as in Central and South Europe. While it has been reported from Iowa, Illinois, Kansas, Minnesota, Mississippi, Nebraska, New Jersey, Ohio, and Texas, according to Clinton (1904, p. 393), it is fortunately still quite rare in this country. Clinton states that it was probably introduced into the United States with importations of sorghum seed from Europe. This seems quite possible in considering Kellerman and Swingle's (1890, p. 159) original note on its occurrence in this country, where it is noted that it first occurred in New Jersey on Amber sorgo (sweet sorghum). In Kansas it was first noticed on "Red Liberian" (sumac)

[1] Citations to literature in parentheses refer to "Literature cited," p. 369–371.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. II, No. 5
Aug. 15, 1914
G—29

sorgo (Failyer and Willard, 1890, p. 145), which would suggest Africa as its source.

There appear to be three distinct forms of smut (Pl. XXXII, fig. 1) affecting the sorghum crop in America (Potter, 1912): *Sphacelotheca cruenta* (Kühn), *Sphacelotheca sorghi* (Link) Clint., and *Sorosporium reilianum* (Kühn) McAlp., the head smut (Pl. XXXII, fig 2). Of these the last-named alone has consistently resisted efforts to prevent its spread, though all known methods for the prevention of cereal smuts have been tried. The serious occurrence of the disease has been observed to be confined at present to the Texas Panhandle. For this reason the investigations, begun in 1907 by Dr. E. M. Freeman and continued after 1909 by the writer,[1] have been carried out chiefly at Amarillo, Tex., with plantings at other points for comparison. This work has been supplemented by studies in the greenhouse and laboratory at Washington, D. C.

SYNONYMY

The head smut of sorghum was first noted by Julius Kühn (1875), who described it from a specimen sent to him from Egypt by Dr. Reil in 1868.[2] The mistake he made in describing the spores as smooth was repeated by Passerini (1876) when he described the form of maize. The echinulations are often obscure, however, unless the spores are quite mature and dry. Brefeld (1883, p. 94) describes them as almost smooth.

Saccardo (1876) and de Toni (1888) described this smut as showing an aggregation of spores suggestive of Sorosporium, as did also Norton (1896, p. 233). Busse (1904, p. 381) suggests in this connection, as Brefeld (1883, p. 171) did earlier, that possibly the genus Sorosporium should not be retained. Busse notes and figures the characteristic spore aggregates, but states that this smut is intermediate in this respect between Ustilago and Sorosporium. According to Dietel (1900, p. 7), the two genera are not sharply distinguishable. Although the spores are rather loosely bound together in this species, McAlpine (1910a, p. 181) has recently placed it in the genus Sorosporium. Under the present artificial system necessitated by a lack of adequate knowledge of the natural relationships

[1] The author wishes to acknowledge the advice and assistance of Mr. E. C. Johnson, who was in charge of the cereal-disease work from 1908 to 1912, inclusive, during which time most of the work here presented was done. Considerable assistance has also been given by various officials at the stations where the work was performed, among whom Dr. E. M. Freeman should be especially mentioned.

[2] "*Ustilago Reiliana* Kühn in litt. U. sporis larvibus, subglobusis, crassiusculis (10, 4 Mikr. inter et 11, 3 Mikr. diamet. variantib.) semipellucidis, brunneis; paniculam totam contractam et obvolutam et abortivam corrumpens. Crescit in Sorgho vulgari." Rabenhorst's Fungi Europaei Exsiccati, No. 1998.

The name given by Kühn is still retained by European mycologists. Its synonymy follows:

Ustilago reiliana Kühn, 1875, *in* Rabenh., Fungi Europ. Exs., ed. nova, s. 2, cent. 20, no. 1998.

Ustilago reiliana, forma *zeae*, Pass., 1876, *in* Rabenh., Fungi Europ. Exs., ed. nova, s. 2, cent. 1 (resp. cent. 21), no. 2096.

Ustilago pulveracea Cooke, 1876, *in* Grevillea, v. 4, no. 31, p. 115, pl. 63.

Cintractia reiliana Clint., 1900, Ill. Agr. Exp. Sta. Bul. 57, p. 346.

Ustilago (*Cintractia*) *reiliana* forma *follicola* Kellerm., 1900, *in* Ohio Nat., v. 1, no. 1, p. 9, pl. 2.

Sphacelotheca reiliana Clint., 1902, *in* Jour. Mycol., v. 8, no. 63, p. 141.

Sorosporium reilianum McAlp., 1910, Smuts of Austral., p. 181.

in this group this classification seems proper in view of his illustration (pl. 30, fig. 37) and of our Plate XXXIII. From these it is evident that the spores, as they occur aggregated into irregular groups, are so formed in the sorus, for the spore balls are found before the spores are mature or even before the latter are differentiated—i. e., while the fungus is still in the hyphal stage.[1]

GROWTH IN ARTIFICIAL CULTURES

The recent work of Appel and Riehm (1911, p. 346, pl. 4[2]) has again emphasized the fact, first established by Brefeld, that the smuts can be cultivated on artificial media in their saprophytic stages. Similar work with this organism has been found difficult on account of trouble in collecting spore material free from contamination and thoroughly germinable. Indeed, the writer has rarely succeeded in getting over 15 per cent of the spores to germinate. The large, open sorus, moist with the saccharin juices of the host, gathers yeasts, molds, and bacteria, which are very troublesome, particularly in liquid cultures. These were attempted repeatedly in several different seasons and at various times of the year, but with only slight and irregular germinations, no matter what the age, source, or condition of the spores. Cane-sugar solutions were largely used, as well as distilled water, rain water, tap water, soil decoctions, sorghum sap, beef bouillon, decoctions of carrots and of prunes, Uschinsky's solution, and Cohn's solution, the last named being also tried in the modified form used by Hitchcock and Norton (1896, p. 200) in their work with this smut. The temperatures were not controlled or recorded in most cases.

With solid media, however, the isolation of the spores found germinating was accomplished by transplanting them with glass hairs under the binocular microscope to sterile poured plates, where their development into conidial colonies was watched under the microscope. Plates seeded thinly enough to contain few contaminations would so seldom show any germinating spores that transplanting from a thickly seeded plate proved to be the only practicable method of isolating, since the head-smut colonies developed so slowly at ordinary temperatures (over a week was required after germination for the colony to become visible to the naked eye) that the plates would be obscured by other organisms long before the smut could be isolated in the usual way. Moreover, the method employed made it certain that the conidia thus obtained in pure culture were not those of some contaminating yeast. It should be said, however, that since this was done it has been found that the yeast and bac-

[1] The character of the sorus, particularly in the decided deformity of the whole inflorescence, also seems more closely similar to several of the species of Sorosporium than to any of Sphacelotheca as described by Clinton (1904, p. 383–395). Although the observations here presented do not appear to be in accord with the classification given this form in Clinton's monograph, the writer is much indebted to Dr. Clinton for helpful criticism.

[2] Erroneously marked "plate 3."

terial contaminations (not the molds) can be almost entirely eliminated without injury to the spores by treating with copper sulphate (see p. 356–357).

The isolation of the organism gave excellent opportunity for a closer study of its relation to various media and temperatures. Plate XXXIV, fig. 1, shows its growth in about six weeks from transfer on carrot agar at 20° to 23°, 30°, 35°, and 40° C., respectively. At 40° there is no growth. At 35° the growth is very slight, light brown in color, and much attenuated. A culture at 32.5° C. grew poorly, and those at higher temperatures were eventually killed, for they did not grow on being removed from the incubator. The rapid development at 30° indicates that this is very near the optimum temperature for the organism, and this is borne out by the studies of germination given in Table I.

TABLE I.—*Germination of spores of the head-smut organism at various temperaiures*

Serial No.	Date of test.	Temperature.	Duration of test.	Germination.
	1912.	°C.	*Days.*	*Per cent.*
1	Dec. 16	29–31	3	6.0
2	...do.....	[a]20–21	3	2.0
3	...do.....	16–20	2	.2
4	...do.....	17	3	.2±
5	...do.....	14.5	3	0
6	...do.....	12	3	0
7	...do.....	8.5	3	0
8	...do.....	7.5	3	0
9	...do.....	4	3	0
10	...do.....	1	3	0
	1913.			
11	Jan. 8	40	3	0
12	...do.....	37.5	3	0
13	...do.....	35	3	0
14	...do.....	32.5	3	1.5
15	...do.....	30	3	7.9
16	...do.....	[a]23–25	3	3.0
17	...do.....	[a]20–23	3	1.0
18	...do.....	18–20	3	2.0
19	...do.....	20–23	3	5.0
20	Mar. 18	27	8	4.0
21	...do.....	17	8	.4
22	...do.....	9	8	0
23	...do.....	23	8	2.0+
24	Mar. 19	27	7	13.1
25	...do.....	17	7	1.0

[a] All but these were incubated in the dark.

These germinations were made in carrot-agar plates with material collected at Amarillo, Tex., in September, 1911, from Red Amber sorgo, except the last two, which were from kafir grown in 1912. From Nos. 11 to 19, inclusive, the number of spores counted in each case was 200; for the rest of the tests the count was not recorded except as follows: No. 20, 1,000; No. 23, 500; No. 24, 541; and No. 25, 818.

In respect to its optimum temperature, then, the head smut is quite unlike those smuts which infect chiefly from seed-borne spores.[1] It is, on the other hand, closely similar to those infecting intraseminally—i. e., the loose smuts of barley and wheat (Appel and Riehm, 1911, p. 364)—and also seems to resemble corn smut, *Ustilago zeae* (Beckm.) Ung., which, while infecting extraseminally, has a late period of infection and shows a more or less localized development. Preliminary observations on corn smut indicating a similar relatively high optimum temperature were made at the same time as Nos. 11 to 19, inclusive, in Table 1; and it is this analogy, rather than that with the loose smuts, which has been supported by the evidence of inoculations and other experiments, presented later.

The fact that the head smut is indigenous to a host from subtropical climates should also be pointed out in this connection. At low temperatures, however, the organism can not be said to be injured, although it grows very slowly, if at all. Even severe freezing does not kill it. Both the spores and conidia have been frozen at St. Paul, Minn., at outdoor temperatures which reached a minimum of −26° C., in both a wet and dry condition, and some were still found to be viable, though frozen for over three weeks. Similar tests at Amarillo, Tex., and at Washington, D. C., were generally confirmatory of these results, although much weathering sometimes appeared to destroy viability.

The writer has not found the spores readily germinable after several years, as did Brefeld (1883, p. 95). Furthermore, the conidia have not survived periods of drying, lasting from four to eight months at ordinary summer temperatures. The method used for determining the latter was to smear some cover glasses with conidia from carrot-agar culture and leave in a Petri dish or culture tube for the period mentioned before transferring to a culture medium for test of viability.

The organism has been found to develop well on malt extract and beerwort agars—perhaps even better than on carrot agar. A synthetic dextrose agar is also favorable. Plate XXXIV, figs. 2 and 3, shows the characteristic, rugose conidial growth. Carrot agar gives a more rapid growth, but the darkened central area of the culture shown in Plate XXXIV, fig. 3, is becoming brown. This may be caused by differences in drying or by the influence of contaminations near it in the plate. A malt extract prepared from germinated Amber sorgo seed was tried, but did not prove to be as favorable a medium as the others. On a 3 per cent cane-sugar agar the growth was scant. Gelatin is liquefied readily. While the organism grows well in 1 per cent peptonized (1 per cent of peptone) solutions of saccharose, lactose, levulose, dextrose, and maltose,

[1] See Herzberg (1895, p. 21) on *Ustilago avenae*. Dr. H. B. Humphrey, at present pathologist in the Office of Cereal Investigations, has found in unpublished experiments that *Tilletia tritici* has an optimum temperature of very close to 20° C.

it does not ferment any of them. Spores, or decidedly sporelike bodies [1] (Pl. XXXIV, fig. 4), are frequently formed in liquid cultures, which then show the brown color characteristic of the resting stage. These may also be found occasionally in agar cultures. They are usually undersized (7.5 to 12μ) and show only traces of echinulations. Their germination has not been observed. In the upper part of the figure (Pl. XXXIV, fig. 4) are shown some of these artifically grown chlamydospores (on the left) with natural spores (on the right) for comparison. Below are shown chains of spores and examples of peculiar formations which are suggestive of the involution forms in many bacteria.

FLORAL ALTERATIONS

A peculiar reaction between this parasite and the host manifests itself by a vegetative stimulus to the host, not only in the vegetative parts but also in the inflorescence.

The parasite of head smut does not always develop a sorus on an infected culm, but frequently causes a floral sterility (Pl. XXXV, fig. 1) which develops at times into a peculiar proliferation of the panicle (Pl. XXXV, fig. 2). This phenomenon, in the tassels of maize, has already been noted and figured by Hitchcock and Norton (1896, p. 199). In extreme cases of this sort in sorghum (Pl. XXXV, fig. 2) the ovary and stamens entirely disappear and the growth takes the form of a complete individuation in the place of each flower; a tiny culm, with leaves, nodes, and rudimentary panicle, shoots up from the head almost as if in an effort to escape the parasite. The hyphæ of the latter were found in one instance to have penetrated the tissues of the phyllomorphic or almost phytomorphic flower (Pl. XXXVI). They are distinctly shown in the illustration as darkly stained threads in the upper part of the panicle and in the bud at its base. In some of the parenchymatous tissue the nuclei are abnormal and have taken the stain like the hyphæ. A number of other flowers less strongly proliferated were examined and found to contain no hyphæ. It may be concluded from this that the change is probably caused by alterations in nutrition processes, especially since a somewhat similar though less pronounced phyllomorphism has been observed in districts where the head smut does not occur, as at Arlington, Va. (Kusano, 1911).

Where the smut occurs commonly, however, this proliferation of the inflorescence is very characteristic and furnishes a more ready means of distinguishing the infected plants than the presence of the sori themselves. Indeed, of 125 plants of Red Amber sorgo examined in three different seasons (1910, 1911, and 1912), mostly at Amarillo, only two

[1] Brefeld (1883, p. 158) obtained the spores of *Tilletia tritici* in artificial culture and Busse (1904, p. 375) has done so with another sorghum smut, *Ustilago cruenta* Kühn. He did not culture the head smut, doubtless because of the interference of contaminations which he mentions (p. 377). Griss (1902, p. 219) has described spore formation in *U. zeae* in cultures. Herzberg (1895, p. 7) does not consider them analogous to those formed on the host, although he germinated some of them in the case of *U. tritici*.

were found to be wholly smutted—i. e., producing spores in every head. Infected plants of this variety almost always have some normal culms, although the number of these varies greatly with the season. Of the 125 plants examined, 64, or more than 50 per cent, produced one or more culms with normal panicles. An infected culm may bear a normal head, but this is rare. Usually such a culm bears no seed, and there is almost always some degree of abnormality in evidence, the glumes becoming elongated and either decolorized or of a greenish hue.

INFECTION OF NODAL BRANCHES

Along with these floral changes there usually occurs an abnormal tendency to branch. Indeed, the development of the buds, which occur alternately on opposite sides of the culm at each node, much as in other Gramineae (Hackel, 1887, p. 3), is often the only positive evidence of the infection, since the resulting branches usually bear sori. This phenomenon has led Busse (1904, p. 386–392)[1] to consider the infection of a branch to take place from hyphæ within the node, growing up through the tissue of the sheath at the time the bud begins to develop, and he evidently concludes (p. 391) that these nodal buds are not infected until they begin to grow out into branches. The histological data given in support of his view seem inadequate to establish, beyond a question, his identification of smut hyphæ in the lesions which sometimes occur in the sheath over the swollen buds. The present investigation has shown, too, that these buds become infected without reference to their development into branches and that there is a peculiar regularity about the infection even when some of the branches are missed.

Forty culms from 15 infected plants of Red Amber sorgo (S. P. I. No. 17548) grown at Amarillo were dissected and studied for the occurrence of the parasite in the nodal buds, and the results are summarized in figure 1. The material was killed and fixed with aceto-alcohol (Carnoy's fluid), a mixture of one-third of glacial acetic acid and two-thirds of commercial alcohol, for periods varying from 2 to 24 hours. It was then rinsed in two or three changes of 70 per cent alcohol and kept in this until embedded in paraffin in the laboratory at Washington, D. C. All the buds from a single culm were prepared and kept together in one vial and were distinguished from each other by cutting them into different shapes, which were sketched into a record showing their position on the culm.

The oft-recurring difficulty in definitely differentiating between the host and parasite by staining methods was encountered in this work. After experimentation it was found that this organism is Gram-positive under most conditions, and with a counterstain of eosin in clove oil a very

[1] Busse (1904, p. 392) says, "Ich nehme an, dass die Infektion nicht direkt, sondern auf dem Umwege über die mit dem Stengel organische verbundene Hauptsprossscheide zu stande kommt." *See also* his Pl. V, figs. 15, 18, 18c, and 19.

sharp contrast was obtained. This proved to be a quick, convenient method, and the stain is fairly permanent if the clove oil is carefully washed off with xylol before mounting in balsam.

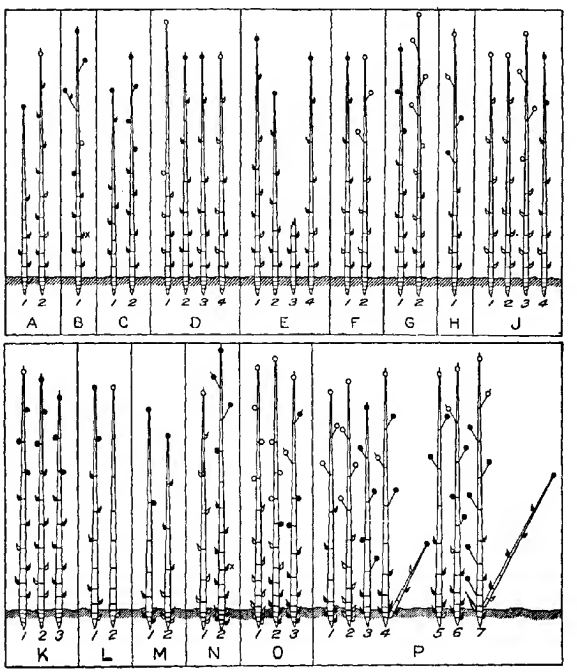


FIG. 1.—Diagrammatic representation of the occurrence of infection in the nodal buds or branches of several sorghum plants.

In figure 1 each plant is designated by a letter and its culms by numerals. The culms are represented with nodes and with branches where they occurred, but without leaves, sheaths, or roots. The growth at each node is represented as follows: A bud which has developed a panicle, either directly evident, as in the main inflorescence, or rudimentary and discovered in dissecting, is represented by a circle, while buds developed to a lesser degree are represented by a subovate symbol. Those showing spore development or, upon microscopic examination, the presence of the hyphæ of the parasite, are shown in solid black, while those which were normal are in outline. In cases where the panicle was not completely parasitized or where the inflorescence, while showing no spore formation, was wholly or partially sterile, the culm is represented as extending through it, the presence or absence of spore formation being indicated as above. When no growth is represented at a node, it signifies that the bud was lost in handling or that for some other reason it was not examined.

All the plants represented in figure 1, except O, were dissected in the early autumn of 1910 at Amarillo. Plant O was one of a number prepared in 1911. In plants A to K, inclusive, no buds were taken from below the surface of the ground. In all cases, however, the exact position of the ground line was not recorded, but has been assumed. The buds on the suckers shown in plant P were not necessarily situated as shown, since they were too small to differentiate by the method used. Culm P_3 also bore a sucker at the first node, on which three buds were infected and three apparently undiseased, the apical bud being lost.

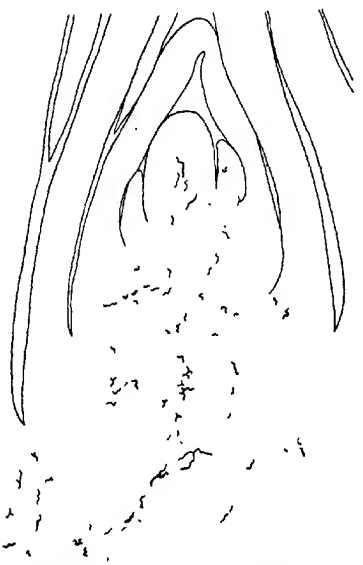


FIG. 2.—Diagram of Plate XXXVII, figure 1, showing the position of the hyphæ.

An examination of the diagrams reveals the fact that most of the culms were but partially infected. A particularly noticeable feature is that when only a few of the buds were missed by the parasite they occurred neither at consecutive nodes nor yet irregularly, but almost without exception included only such as were on the same side of the culm. This is well illustrated in culms A_2, D_3, E_1, E_4, F_1, J_4, and L_1. In the same way, if only a few of the buds were involved in the infection, they, too, were usually on the same side of the culm and at the base of the plant, as seen in culms D_1, D_4, and F_2. The basal portion sometimes escaped (as in culms K_2 and M_2), and occasionally the top grew away from the

parasite (as in culms O2, O3, P1, P5, P6, and P7), though usually remaining sterile. Thus, the plant is seen to have been infected only in such of the buds as were developed from a definite section of the original meristem. The few irregularities (culms G2, H1, K1, O2, and P6) can not be said necessarily to conflict with this interpretation, but were probably the result of unusual developments, such as a double infection, or, perhaps, of errors in technique or records in repeatedly handling these 300 or more buds. It seems certain that the dominance of cases showing regularity of infection can not be due to error.

Plate XXXVII illustrates the appearance of the hyphæ in two of these nodal buds. The two buds in question are marked by a cross in text figure 1. In Plate XXXVII, figure 1, the host tissue was stained more deeply than in the other, and the hyphæ, which are intercellular, do not show as well, particularly those not exactly in focus. Text figure 2 will assist in locating such as are discernible in Plate XXXVII. It should be noticed that in this section the hyphæ are seen mostly in the tissues on the left, while in the other nearly all of them are on the right. Such an arrangement doubtless occurred in the buds from which such infections developed as are shown in culms A2, D4, E1, F1, etc.

It is apparent that no assumption of the occurrence of the primary infection at or near the maturity of the host can explain the regularities of the infection phenomena usually found in these buds without also assuming an improbable spread of the infection in the mature tissues of the host. The nodal branches were evidently infected early, when the buds formed, if at all. As Brefeld (1895, p. 47, 84) observed in connection with his work on infection with *Ustilago cruenta*, the sorghum plant grows very slowly at first for a period of about four weeks or more. It was during this time, then, while the meristem, at least in each culm, was confined to a comparatively small compass, that the spread of the infection must have proceeded in such a way as to determine its later development in these plants.

LIFE HISTORY OF THE PARASITE

PREVIOUS WORK

That the head smut infects its host in the early seedling stage has been the general assumption as to its life history, although the results of inoculations performed by investigators would seem to have given doubtful support to the idea. Brefeld (1883, p. 94) states that Kühn, who named this parasite, obtained a double, artificial infection with this smut and *Ustilago cruenta*. Passerini (1877, p. 236) says he was able to reproduce the head smut on maize, but not on sorghum. W. A. Kellerman (1891, p. 98, 101) produced slight infection in greenhouse and field experiments by inoculating the seed. Later (1900a, p. 9)[1] he

[1] See "Literature cited" for notes published in 1898; with K. F. Kellerman in 1899; and with O. E. Jennings, reporting further negative results, in 1902.

produced it also on maize and described the form *foliicola*. While he states (1900, p. 18) that infection from seed-borne spores takes place and that, therefore, seed treatment with fungicides is of value, he had, like Passerini, produced the disease, in the field, only on maize and in very small quantities. Clinton (1900, p. 347) also failed to produce any infection by inoculations of the seed and young plants. Hori (1907, p. 163, 166) reports entirely negative results from inoculations, but claims that a hot-water treatment has been shown to prevent the disease. McAlpine (1910, p. 296) produced infection in a single maize plant by seed inoculation and on this basis recommended seed treatment with copper-sulphate solution as a preventive. Johnston (1910 or 1910a, p. 44) has also recommended seed treatments, and this Australian idea has been copied by Mundy (1910, p. 4) in South Africa.

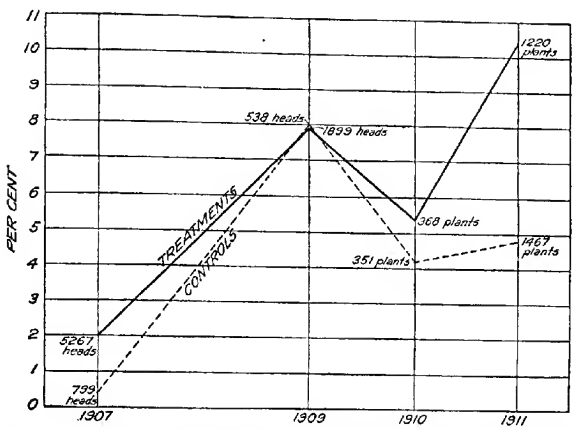


FIG. 3.—Curves summarizing for different years the percentages of infection in plantings of sorgo after all hot-water treatments and in control plantings.

The early inoculation experiments of the Office of Cereal Investigations, involving about a thousand plants of different varieties (including kafir and sorgo) in the field at Amarillo, gave results similar to those cited above—i. e., little or no infection resulted from the presence of an abundance of spores on the seed.

SEED AND SPORE TREATMENTS

In full accord with the negative results of these inoculations our experiments have conclusively shown that the most severe treatments of the seed, though carefully performed, do not prevent the attack of the parasite. These treatments have involved some 35,000 or more plants, of which about two-thirds were in tests of thermal methods, the

rest of the tests being performed with fungicides. For the latter, formalin, copper sulphate, cresol, and potassium sulphid were tried. Kafir, broom corn, and sorgo were used, and of these the first two developed so little infection that the results were of no significance.

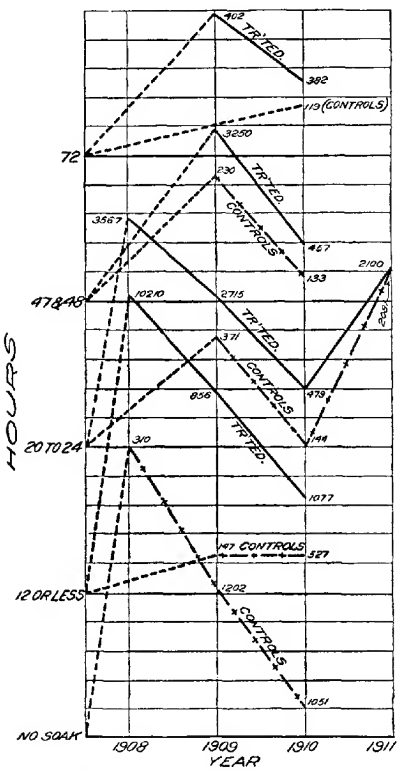


FIG. 4.—Curves summarizing for different years the percentages of infection: First, in plantings of Red Amber sorgo after modified hot-water treatments at all temperatures and of all durations, but after presoakings of various duration; and, second, in control plantings (not treated with hot water).

With the more susceptible sorgos (chiefly the Red Amber variety), however, quite heavy infections occurred in some seasons. The important features of the results are brought out in the summaries presented in figures 3 to 7, inclusive. The first and last of these figures present results obtained with several varieties of sorgo, the one being a summary of treatments performed with hot water without presoaking and the other a summary of the whole work on seed treatments, including both thermal and chemical methods. The three others (figs. 4, 5, and 6) show the results of modified hot-water treatments [1] of Red Amber sorgo (S. P. I. No. 17548) according to the three elements of the treatment: figure 4, according to the length of presoaking given the seed; figure 5, according to the duration of the hot-water treatment; and figure 6, according to its temperature.

[1] This method was originated by Jensen (1888). See Freeman and Johnson (1909) and Appel and Riehm (1911). Tepid water for presoaking was tried in a few of these treatments of sorghum, but without any difference in results.

In summarizing the results for constructing these curves, the duration of presoaking in the modified treatments and the duration and temperature of treatments have been approximated in several instances in order to bring all of them to intervals of 12 hours of presoaking, 5 minutes in duration of treatment, or 2 degrees in temperature. The results of treatments performed in 1909 and previously were recorded by counting heads, while subsequently they were recorded by noting the number of plants. These numbers are given at each point in the curves.

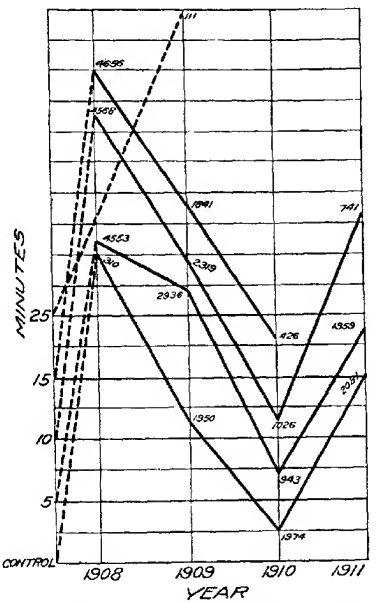


FIG. 5.—Curves summarizing for different years the percentages of infection in plantings of Red Amber sorgo after modified hot-water treatments at all temperatures and of all presoakings, but after treatments of various durations.

It is evident from the curves in all these illustrations not only that the treatments in no way reduced the amount of infection, but also that, regardless of treatment, the percentage of smutted plants occurring varied consistently with the season. Indeed, the curves in figures 4, 5, and 6 proved to be, with scarcely an exception,[1] so nearly alike for all the treatments that they could not well be drawn to the same coordinates. They are therefore separated, and each curve is continued by a broken line to the axis of the coordinates to which it is drawn, each interval therefrom representing 1 per cent of infection.

While it is true that infection by any phytopathogenic organism would vary with seasonal conditions regardless of the exact features of its life history, an added significance in these curves is found when it is noted that

[1] The only case in which these curves do not very nearly coincide is in the 54° C. treatments of 1909 (fig. 6). In this case there were but 151 heads on which to base the 1909 figure, this being so small that the result, which is characteristic of the irregular occurrence of the infection at Amarillo, is plainly dependent upon some peculiar minor factor, such as a variation in soil conditions, rather than upon the season. It is certainly not owing to the treatment of the seed.

the plantings at the Cereal Field Station at Amarillo were on new land both in 1907 and 1910. This station was established in 1907 and removed to another situation, also at Amarillo, at the latter date. In view of the fact that the presence of the organism has proved to be so salient a factor, as established by seed exchange and inoculation experiments, presented later, it would seem proper to attribute the light infection in 1907 and 1910 to the relative scarcity of the infective stage of the organism in the virgin soil. The large increase in 1908 was probably due to the proximity to the station of an old field which grew a rather badly smutted crop of sorgo each year. The decrease in 1909 was doubtless caused by drought, scarcely half of the crop being headed.

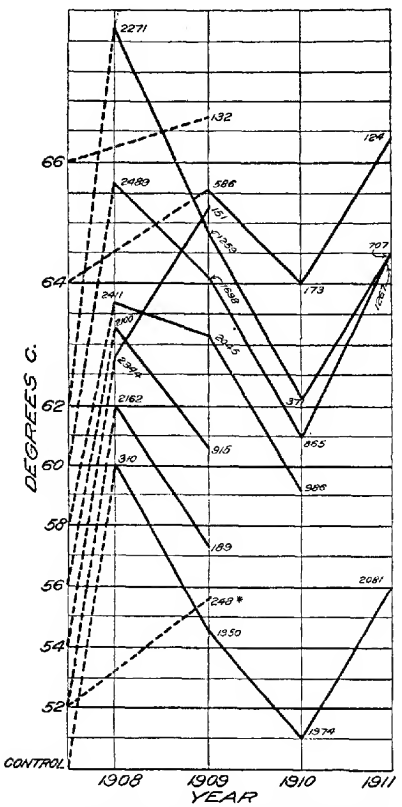


FIG. 6.—Curves summarizing for different years the percentages of infection in plantings of Red Amber sorgo after modified hot-water treatments of all durations of presoaking and treatment, but at various temperatures.

The inevitable conclusion from these experiments is that infection commonly takes place from some other source than seed-borne spores. This conclusion has been supported by tests of the effect of some of these treatments on the viability of the spores. Tables II and III present the results of these tests. They were somewhat obscured by the comparatively sparse germination so characteristic of these spores and by the development of the contaminations contained in the untreated spore material used in seeding check plates. The treatments with hot water were carried out, mostly on March 10, 1913, as follows.

Spores from Red Amber sorgo of the crop of 1911 were used in most cases. Before treatment they were thoroughly wet by shaking with distilled water. The dirt and foreign material were removed by centrifuging, and later the single spores were separated from the spore balls by the same method. In Table II, Nos. 1 to 14 and 29 to 34, inclusive, separated spores were used, while spore balls were used for the other treatments, except the last two, which were mixed. With a wire loop the spores or spore balls were transferred from the wet mass at the bottom of the centrifuge tube to tubes of water, which were then placed in the thermal bath. At the end of the period of treatment a portion of the spores in suspension was poured or pipetted out of the tube into melted agar at 43° C., in which they were shaken up and were then poured into a Petri dish. This portion was incubated at 27° to 28° C. and was examined from time to time under the microscope for germinating spores.

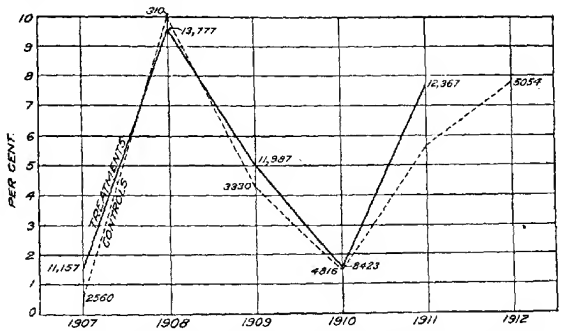


FIG. 5.—Curves summarizing for different years the percentages of infection: First, in plantings of sorgo after all seed treatments; and, second, in control plantings.

In the later treatments at 60° C. (Table II, Nos. 29 to 34, inclusive) the spores were subjected to the hot-water bath in the tubes of melted agar, thus avoiding the subsequent transfer. The first method would appear to give more chance for error, and to this is due, perhaps, the slight survival noted after rather severe treatments.

In Table II it is seen that moist heat is fatal within the upper range of temperatures used in the seed treatments (see fig. 6), and even dry heat seems injurious to the spores of this smut (Table II, Nos. 35 and 36). The plantings from hot-water and modified hot-water treatments of the seed showed a field infection in no way correlated with the thermal death point of the spores. About 24,000 plants grown from seed treated according to the latter method showed an infection of 5.9 per cent as against 3.1 per cent in about 3,500 plants grown from untreated seed. Over 15,000 of the plants from treated seed were of the Red Amber sorgo variety, which showed 6.5 per cent of smutted plants as against 4.2 per cent in the controls.

TABLE II.—*Results showing the effect of various hot-water and modified hot-water treatments on the viability of the spores of the head-smut organism*

Serial No.	Treatment.			Duration of test.	Number counted.	Germination.
	Duration.	Temperature.	Duration of presoaking.			
	Min.	*° C.*	*Hours.*	*Days.*		*Per cent.*
1	.....	Control.	(a)	2	1,225	3.8
				3	535	4.5
				3	.....	Trace.
2	10	55	(a)	4	.....	Trace.
				3	1,500	0
3	20	55	(a)	4	.....	0
				3	3,000	.1
4	10	60	(a)	5	.....	No increase.
				3	3,000	0
5	20	60	(a)	5	.....	0
6	10	65	(a)	4	2,500	.04
7	20	65	(a)	4	5,000	0
8	.....	Control.	6	2	1,046	9.6
				2	600	0
9	10	55	6	3	.....	1—
				4	400	3
				2	.....	2
10	20	55	6	3	.....	Trace.
				4	700	2.9
11	10	60	8	3	3,000	.03
				5	.....	.5—
12	20	60	8	3	4,000	0
				5	5,000	.02
13	10	65	8½	4	3,000	.33
14	20	65	8½	4	5,000	.04
				2	572	1.6
15	.....	Control.	(a)	3	.....	5+
				4	.....	No increase.
				3	400	0
16	10	55	(a)	4	.....	Trace.
				3	500	0
17	20	55	(a)	4	.....	0
				3	550	0
18	10	60	(a)	5	.....	0
				3	.....	0
19	20	60	(a)	5	.....	Trace.
20	10	65	(a)	4	.....	Trace.
21	20	65	(a)	4	.....	Trace.
22	.....	Control.	6	2	503	19.7
				2	402	Slight.
23	10	55	6	3	.....	2+
				4	100	30
				2	650	0
24	20	55	6	3	.....	0
				4	.....	0
				3	.....	0
25	10	60	8	5	.....	0
				3	.....	0
26	20	60	8	5	.....	0
27	10	65	8½	4	600	.17
28	20	65	8½	4	522	0
				2	1,000	4
29	.....	Control.	(a)	8	.....	No increase.
				2	.....	0
30	10	60	(a)	8	.....	0

a Not soaked.

TABLE II.—*Results showing the effect of various hot-water and modified hot-water treatments on the viability of the spores of the head-smut organism*—Continued

Serial No.	Treatment.			Duration of test.	Number counted.	Germination.
	Duration.	Temperature.	Duration of presoaking.			
	Min.	*° C.*	*Hours.*	*Days.*		*Per cent.*
31	20	60	(a)	2	.....	0
				8	.....	0
32	.....	Control.	6	2	950	4.5
				8	.....	No increase.
33	10	60	6	2	.....	0
				8	.....	0
34	20	60	6	2	.....	0
				8	.....	0
35		Control.	(a)	3	.....	13.1
36	5	70	.....	2	.....	Slight.
		Dry heat.		6	.....	Slight.

[a] Not soaked.

In the tests of the effect of fungicides on the spores the solutions of different strengths, including water for control, were prepared at a temperature of 22° to 23° C. and placed in culture tubes. The spore material was prepared as for the thermal tests and transferred to the tubes in the same way. The culture tubes were then thoroughly shaken. At the end of the period indicated in the tables the tubes were again agitated and with a pipette 5 c. c. were removed from each to the centrifuge tubes, which were immediately filled with water. The spores being thrown down by centrifuging, the water was poured off and the tubes refilled, this rinsing being repeated four or five times. The last rinsing water from the strongest treatment was poured on to the control, which was then recentrifuged, to make certain that the rinsing had removed the treating solutions effectively. Further water being added, enough of the suspension of spores was poured into a tube of melted carrot agar at about 43° C. to make a thickly seeded plate. The plate was poured immediately, incubated, and examined as in the other tests.

In the work with copper sulphate, solutions equivalent to from 0.35 per cent to 2.52 per cent of $CuSO_4$ were used in treatments of sorgo seed, some of which had had the glumes removed before treatment. In one series (1907) a 17-hour soak with the weakest of these solutions gave plants with 2.3 per cent infection as against none in the controls, while in another series (1911), using seed without glumes, a 10-minute treatment with the strongest solution resulted in 13.1 per cent of infected plants as against 2.8 per cent in the controls.[1] Other treat-

[1] The fact that all of these treatment experiments, except the modified hot-water treatments, were also infected by *Sphacelotheca sorghi* seems to have had a peculiar bearing on these comparative percentages. In nearly all cases a considerably larger amount of head smut occurred in the treated lots than in the controls, which, not having been treated, were heavily infected by the kernel smut. The latter seemed to get the start of the head smut and prevent its development, for no case of evident double infection, as was observed by Busse (1904, p. 381), was found. Thus, in the various treatments of Red Amber sorgo carried out in 1911 with formalin, cresol, copper sulphate, and hot water, 24 treated lots containing 3,616 plants averaged 10 per cent of head-smut and 2.6 per cent of kernel-smut infection, while 15 lots (3,081 plants), untreated or unsuccessfully treated for the kernel smut, contained 5.8 per cent of head smut as against an infection of 29 per cent by the kernel smut. One lot with 62.3 per cent of kernel smut had 3.3 per cent of head smut; in another the percentages were 57.1 and 1.8, respectively. This phenomenon seems to have an adequate explanation in the comparatively late period of infection shown for the head smut (see p. 365).

ments involving, with controls, some 3,500 plants, were equally ineffective and inconsistent in their results.

But the spores are not at all injured by even more severe treatments. Table III, Nos. 11 to 18, inclusive, gives the results of these tests performed with the spores on March 7, 1913. It might even be said that the development of conidia proceeded better in the plates containing treated spores, probably on account of the absence of contaminations, these being for the most part killed by the treatment. It is possible that a longer treatment, even with less concentrated solutions, would have killed the spores (Herzberg, 1895, p. 30), but this would be likely to injure the seed as well.

TABLE III.—*Results showing the effect of various formalin and copper-sulphate treatments on the viability of the spores of the head-smut organism*

Serial No.	Treatment.		Duration of test.	Number counted.	Germination.
	Duration.	Method.			
	Min.		*Days.*		*Per cent.*
1	.....	Control, not treated....	3	681	4.1
			7	.....	No increase.
2	34	0.16 per cent formaldehyde solution	3	.....	0
			7	1,000	1.6
3	33	0.24 per cent formaldehyde solution	3	.....	0
			7	.....	0
4	60	0.16 per cent formaldehyde solution	3	.....	0
			7	.....	0
5	60	0.24 per cent formaldehyde solution	3	.....	0
			7	.....	0
6	.....	Control, not treated....	3	541	13.1
			7	.....	No increase.
7	60	0.16 per cent formaldehyde solution	3	.....	0
			7	.....	Trace(?).
8	.....	Control, not treated....	3	.....	Slight.
			7	.....	Slight.
9	60	0.16 per cent formaldehyde solution	3	.....	0
			7	.....	0
10	60	0.24 per cent formaldehyde solution	3	.....	0
			7	.....	0
11	.....	Control, soaked 30 minutes	28	1,068	5.2
12	30	0.76 per cent copper-sulphate[a] solution...	28	1,044	5.0
13	30	1.52 per cent copper-sulphate solution....	28	1,034	5.2
14	30	2.52 per cent copper-sulphate solution....	28	1,000	4.0
15	.....	Control, soaked 60 minutes	28	1,034	4.5
16	60	0.76 per cent copper-sulphate solution....	28	1,000	1.8
17	60	1.52 per cent copper-sulphate solution....	28	1,000	5.0
18	60	2.52 per cent copper-sulphate solution....	28	1,000	6.0

[a] Copper sulphate = $CuSO_4$.

In an additional test intended to discover the influence of a residual effect of the fungicide after treatment without rinsing, it was found that the presence of a trace of copper sulphate in the medium does not hinder germination. However, Moore and Kellerman (1904, p. 29) found that the toxic action of dilute watery solutions of copper is overcome by certain substances present in most culture media; and Hawkins (1913, p. 68–75) has recently shown that soluble calcium and potassium salts also neutralize the toxicity of copper. The probability that some of these substances were present in the vegetable medium used makes the above test of residual action inconclusive. Nevertheless, Dandeno (1908, p. 60) states that *Ustilago zeae* germinates readily in a N/2,048 watery solution of copper sulphate. Copper fungicides do not appear to have a very penetrating action, and the sulphate certainly is not destructive to the head-smut spores within a limited time at ordinary temperatures.

McAlpine (1910, p. 298) found that a 0.12 per cent solution of formaldehyde did not affect the spores inside of 15 minutes. However, the formaldehyde treatment, when sufficiently severe, does kill them, as is shown in Nos. 1 to 10, inclusive, of Table III. These tests were with separate spores, except in the last three, in which spore balls were used.

In spite of this evidence that the spores do not survive one hour's treatment with a 0.16 per cent formaldehyde solution, it was found that seed given this and more severe treatments produced plants with 4.2 per cent of infection in about 3,000 plants (the estimates in the early experiments were by heads) which survived, as against 3.4 per cent in about 2,000 plants from untreated seed. The formalin treatment, therefore, is ineffective, but not because of failure to destroy external seed infection; and it may be said that this is true of the other chemical treatments of the seed, all of which have proved equally ineffective in prevention, even though, like copper sulphate, they may have had no lethal effect upon the spores. Indeed, plants from treated seed seemed the more easily infected in some instances.

FLORAL INOCULATIONS

The evident systemic character of the disease, however, immediately suggested the possible analogy with the loose smuts of barley and wheat. Kellerman's inoculations were made before the possibility of intraseminal infection was realized, and the question occurs, was not Jensen's (1888, p. 61) mistake, in assuming extraseminal infection to have taken place in the case of *Ustilago tritici* when a diseased wheat plant appeared among those he had inoculated, here repeated in the case of sorghum? While the loose character of the spore body and the echinulate spores of the head smut gave added force to the hypothesis of a floral infection, the abundant production of conidia and, as compared with the loose smuts (Appel and Riehm, 1911, p. 363), the prolonged viability of the spores, did not support this analogy.

Numerous floral inoculations, undertaken early in these investigations, also failed to give results supporting this view. These were carried out in several different seasons and at various stages of development in the ovary. Dry spores of the head smut were placed in a paper bag and shaken into the flowers by inverting the bag over a head and shaking thoroughly; sometimes they were placed inside the glumes with a camel's-hair brush. Some of the spores were germinated before applying them, and were sprayed into the flowers with an atomizer either by opening the glumes with forceps, or in the early morning while the plant was still in bloom; some of the heads were not covered, but some were kept covered for a time with paper bags or with a large lamp chimney to keep them moist. This was an extremely difficult matter, however, owing to the high winds and to the consequent rapid rate of evaporation, which, from an open water surface, often exceeds half an inch in 24 hours at Amarillo. While there was occasionally a rather high percentage of infection in the resulting plants, this was not the uniform result of any particular method of inoculaton; nor was it sufficiently large to obviate its explanation by infection of the plants during development in the field, in view of the fact that it did not occur consistently.

ENVIRONMENTAL EXPERIMENTS

In addition to the negative results of inoculations, it was found that seed from the same lots when planted at various points in the United States, or in different seasons at Amarillo, gave very different amounts of infection in the plants produced, while in plants from different lots of seed, grown at the same station, no consistent differences could be observed.

A preliminary experiment was carried out in 1908. The plants were all grown from the same lot of seed, yet those grown at Amarillo were 7.7 per cent smutted and those at Chillicothe, Tex., were 2 per cent smutted, while those at McPherson, Kans., were not affected at all. In 1910 a new series was begun. Plantings were made from two lots of seed at eight different stations, including Amarillo and Chillicothe, Tex., St. Paul, Minn., and Arlington, Va. Of these two lots, that from Chillicothe happened to develop the greater percentage of smutted plants at Amarillo, and the seed grown from it was therefore used for the plantings in 1911. In this and subsequent seasons the intention was to plant at each station seed from each of the places concerned and to use only seed descended from the original lot and grown in consecutive seasons at the same station. This was usually done, but, owing to various mishaps, the plantings at the four stations named were the only ones which were carried completely through the experiment as intended. The data from these four stations thus form a complete series and are summarized in Table IV. They involved in each case from about 150 to 800 plants; usually about 300.

TABLE IV.—*Summary of results showing the influence of locality on the occurrence of head smut*

Seed from—	Percentage of infection at—											
	Amarillo, Tex.			Chillicothe, Tex.			St. Paul, Minn.			Arlington, Va.		
	1910	1911	1912	1910	1911	1912	1910	1911	1912	1910	1911	1912
Amarillo, Tex.	1.6	14.71	10.86	0.14	0	7.14	0	0	0	0	0	0
Chillicothe, Tex.	3	13.09	12.29	.25	0	6.91	0	0	0	0	0	0
St. Paul, Minn.		17.14	1.72		.87	3.52		0	0		0	0
Arlington, Va.		7.56	2.9		2.6	4.46		0	0		0	0

From this it may be seen that no infection occurred at Arlington or at St. Paul. Only a trace of it has ever occurred at St. Paul, except in inoculated plants in 1912. It has not been present at all at the Arlington Experimental Farm or in its immediate vicinity during the three years in question, so far as the writer was able to discover by careful examination. Yet seed from St. Paul produced the highest percentage recorded at Amarillo in 1911, although showing no infection at either Arlington or St. Paul in that year; and seed from Arlington has always produced some smutted plants at the two Texas points. Of the four seed lots used in 1911, the Arlington seed produced the largest number of infected plants at Chillicothe. Moreover, seed grown at either of the two Texas stations never produced smutted plants when grown at the other two stations, although inoculated plants showed abundant infection at St. Paul in 1912 (see Table V, plat E). It should be noted, too, that seed from the same lots used for the Amarillo plantings in 1910 and 1911 were planted at Amarillo in the ensuing years and produced infected plants as follows: 1910 lots, replanted in 1911, 3.8 per cent and 15.6 per cent, respectively; 1911 lots, replanted in 1912, 1.8, 2.7, 0, and 1.8 per cent, respectively. These figures are evidently in no way comparable or consistent with those of the year before, as shown in Table IV.

EXPERIMENTS WITH PROTECTED SEED

As may have been already observed, particularly in connection with the slight irregularities of the curves in figure 6 (see footnote, p. 351), positive conclusions from comparative amounts of infection in small lots of plants at Amarillo are not warranted without consistent results from oft-repeated experiments. However, the appearance of any infection in plants from seed protected from contamination gives additional evidence that the infection is not carried with the seed.

Thus, 177 plants were grown at Amarillo in 1912 from seed produced in the greenhouse at Washington, D. C., on heads which had been covered with transparent paper bags from before flowering until they were thrashed out by hand. One plant (0.6 per cent) was infected. Similarly, 1,669 plants grown in 1912 from seed of 18 heads protected in the

same way but produced in the field at Amarillo in 1911 showed 6.4 per cent of infection. The high winds had torn some of the bags at times, but they were replaced as soon as possible. Moreover, four of them remained intact throughout; yet of the 206 plants grown from the resulting seed, 13, or 6.3 per cent, were infected. This was scarcely less than the average natural field infection in 1912. (See fig. 7.)

This evidence is a particularly strong negation of the floral infection theory, especially when it is noted that the seed lot from the greenhouse in Washington, D. C., produced 8 infected plants out of 18 when the seedlings were artificially inoculated. (See Table V, plat C, No. 5.)

INFECTION EXPERIMENTS

It has been made clear by the results already described that floral infection is not involved in the life history of this parasite and that seed-borne spores, though doubtless functioning at times in distributing the fungus from one district to another, by no means constitute the determining factor in the general field infection. The apparent contradiction in the evidence so far presented—one which has led to many confusing surmises and recommendations in the literature of the subject—remains to be explained by positive evidence of infection from artificial inoculations.

A series of inoculation experiments carried on at Amarillo, Tex., in 1911, duplicated at Amarillo, Tex., at St. Paul, Minn., and at Manhattan, Kans., during the season of 1912, and twice repeated in the greenhouse at Washington, D. C., has confirmed these observations and demonstrated that the presence of the parasite in the soil about the growing seedling is productive of successful infection under any of the conditions prevailing in these various habitats. These results are presented in Table V.

EXPLANATION OF TABLE V

In tabulating these results considerable abbreviation has seemed desirable, and it is herewith explained. When special reference to this explanation is necessary, the abbreviations in Table V are inclosed in parentheses. Under each of the following main headings the column with the same heading in the table is explained.

"Date."—The date given in the column provided is the date of inoculation except in a few cases, usually controls, when it is inclosed in parentheses and indicates the date of planting.

"Seed Lot."—Five different lots of seed, all of the variety Red Amber sorgo (S. P. I. No. 17548), were used and are indicated, in the column provided, by the following symbols:

"I." From the crop of 1910 at Amarillo, Tex. When in parentheses, as "(I)," the seed had the glumes still inclosing it; otherwise it was without them, having been separated in water from the seed which had retained the glumes through the thrashing process.

"II." Seed without glumes (separated in water, as in I); from the crop of 1911 at Amarillo. This seed was treated with a 0.24 per cent formaldehyde solution for one hour, except where the symbol is in parentheses "(II)."

"III." Seed from a head grown at Amarillo in 1911, which had been kept covered with a transparent paper bag from before flowering until thrashed out by hand. The parentheses simply indicate a different head as the source of seed.

"IV." Seed from heads grown in the greenhouse at Washington, D. C., during the winter of 1911–12 and kept covered, as above, from before flowering until thrashed out by hand.

"V." Seed without glumes (separated in water, as in I), from the crop of 1911 at Akron, Colo. Treated with 0.16 per cent formaldehyde solution for 10 minutes after a thorough washing.

"Spore Lot."—The mixed lot of spores used is so indicated; the other five lots, all collected from Red Amber sorgo at Amarillo, are indicated as follows:

"*A*." Collected in the fall of 1910.

"*B*." Collected in September, 1911. The parentheses indicate conidia from cultures first isolated from single spores of this lot (see p. 341) in February, 1912.

"*C*." Collected in the fall of 1912.

"Method."—The methods used in making inoculations are classified—

First, as to the condition of the host plant when inoculated (or planted, in the controls):

"a"=dry seed;

"b"=germinating seed;

"c"=older plants.

Second, as to the character of the inoculating material:

"m"=dry spores;

"n"=suspension of spores in which a few were germinating;

"p"=conidia.

Third, as to the general procedure in inoculating:

"x"=heavy application of a mass of the inoculating material, usually so as to completely cover the seed or seedling when planting it, or, on older plants, to cover the inoculated part;

"y"=lighter application—dusting of dry spores before planting or spray of material in water;

"z"=inoculation of the plat by raking smutted heads into the soil after plowing in the spring. "zz" in plat C, No. 7=inoculation two years in succession, the same plat being used as for plat A, No. 11, the year before.

Fourth, the controls, which were not artificially inoculated, are indicated in this column.

Fifth, special methods in inoculation are indicated by parentheses, as follows:

"bm(x)" in plat A, No. 7=the soil in the opened row was heavily inoculated at planting;

"bn(x)" in plat B, Nos. 1 and 2, plat D, No. 1, and plat E, No. 1, and

"bp(x)" in plat C, Nos. 1 and 2, plat D, No. 2, and plat E, No. 2=both seedling and soil were inoculated;

"cm(x)" in plat C, No. 8, plat D, No. 8, and plat E, No. 5=the spores were placed about the root crown just beneath the surface of the soil;

"cp(x)" in plat E, No. 6=the conidia were taken from carrot-agar culture and smeared on the base of the plant with a flat inoculating needle;

"bm(y)," "bn(y)," and "b(control)" in plat E, Nos. 8, 9, and 10=the ground was thoroughly wet down both before and after planting, the seed only being inoculated;

"bn(y)" in plat A, Nos. 1 and 2=the seed only was inoculated;

"bn(y)" in plat A, Nos. 3 and 4=the soil only in the opened row was inoculated;

"cp(y)" in plat C, No. 9, and plat D, No. 9=conidia were sprayed on the root crown, which was then re-covered with moist earth.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*

PLAT A [a]

[Planted at Amarillo, Tex., in the field; counted Sept. 12, 1911.]

Serial No.	Date.	Seed lot.	Spore lot.	Method.	Total number of plants.	Infection.
						Per cent.
1	May 25	I	Mixed	bn(y)	325	4.9
2	do	(I)	do	bn(y)	383	3.7
3	do	I	do	bn(y)	103	5.8
4	do	(I)	do	bn(y)	70	5.7
5	do	I	do	bmy	165	6.7
6	do	(I)	do	bmy	210	5.3
7	do	I	do	bm(x)	130	34.6
8	May 26	(I)	do	bm(x)	106	23.6
9	May 25	I	do	amy	200	10
10	do	(I)	do	amy	292	4.1
11	(May 23)	I	*A*	az	34	11.7
12	do	(I)	*A*	az	110	5.5
13	May 25	I	..	a, control [b]	196	6.6
14	do	(I)	..	a, control	127	12.6
15	do	I	..	a, control	272	7.3
16	do	(I)	..	a, control	444	3.8

PLAT B [a]

[Planted at Washington, D. C., in pots in the greenhouse of the Department of Agriculture. The even numbers were planted in a 2-inch top dressing of clean sand, while the other pots (odd numbers) contained only potting soil; counted May 16, 1912.]

1	(c)	II	*B*	bn(x)	8	50.0
2	(c)	II	*B*	bn(x)	7	14.3
3 [d]	(c)	II	*B*	bmx	3	100
4	(c)	II	*B*	bmx	3	100
5	(c)	II	..	a, control	5	0
6	(c)	II	..	a, control	7	0
7	(c)	(II)	..	a, control	7	0
8 [d]	(c)	(II)	..	a, control	8	0
9	(e)	II	*B*	bmx	2	50
10	(e)	II	*B*	bmx	1	100

PLAT C [f]

[Planted at Amarillo, Tex., in the field; counted Sept. 7, 1912.]

1	May 28	II	(*B*)	bp(x)0.6 to 1.2 [g]	41	26.8
2	May 30	III	(*B*)	bp(x)	5	0
3	May 28	II	*B*	bmx 0.6 to 1.2 [g]	45	66.6
4	May 30	(III)	*B*	bmx	1	0
5	May 28	IV	*B*	bmx	18	44.4
6	do	II	*B*	amx	102	42.2
7	(May 29)	II	*B*	azz	522	21.45

[a] Inoculations by the author.
[b] Treated for one hour with 0.24 per cent formaldehyde solution.
[c] About Nov. 15, being the date of planting in Nos. 5, 6, 7, and 8.
[d] See Plate XXXV, figure 3.
[e] About Feb. 8.
[f] Inoculations performed by Mr. E. C. Johnson.
[g] The numbers given indicate in centimeters the length of the plumules in Nos. 1 and 3, and the average height of the plants in Nos. 8 and 9. In the latter case the plants were mostly unbranched as yet. In Nos. 10 and 11 the plants were younger than in No. 3.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*—Continued

PLAT C—Continued

Serial No.	Date.	Seed lot.	Spore lot.	Method.	Total number of plants.	Infection.
						Per cent.
8	June 25	II	*B*	cm(x)5[a]	136	2.94
9	do	II	(*B*)	cp(y)5[a]	112	7.1
10	May 29	II	*B*	bmx[a]	45	42.2
11	do	II	*B*[b]	bmx[a]	68	39.7
12	(May 29)	II	..	b, control	112	32.1
13	do	II	..	a, control	148	1.4
14	do	II	..	a, control	185	3.2
15	do	(II)	..	a, control	136	25
16	do	(II)	..	a, control	114	3.5
17	(May 25)	(III)	..	a, control	51	3.9
18	do	III	..	a, control	50	4
19	(May 29)	II	..	a, control[c]	328	1.5
20	do	(II)	..	a, control[c]	268	0

PLAT D[d]

[Planted at Manhattan, Kans., in the field; counted Aug. 30, 1912.]

1	June 4	II	*B*	bn(x)2[e]	1	0
2	do	II	(*B*)	bp(x)2[e]	24	0
3	June 3	II	(*B*)	bp(x)5[e]	1	0
4	June 4	II	*B*	bmx2[e]	24	29.1
5	June 3	II	*B*	bmx3[e]	3	0
6	do	II	*B*	bmx5[e]	1	100
7	June 3–4	II	*B*	amx	50	10
8	June 4	II	*B*	cm(x)	200	0
9	do	II	(*B*)	cp(y)[e]	75	0
10	(June 4)	II	..	a, control	210	0
11	do	II	..	a, control[e]	731	0
12	do	II	..	b6, control[e]	5	0
13	do	II	..	b2, control[e]	26	0

PLAT E[f]

[Planted at St. Paul, Minn., in the field; counted about Sept. 20, 1912.]

1	June 7	II	*B*	bn (x)	49	10.2
2	do	II	(*B*)	bp (x)	(*g*)	0
3	do	II	*B*	bmx	30	26.7
4	do	II	*B*	amx	49	36.7
5	July 5	II	*B*	cm (x)[h]	(*g*)	0
6	do	II	(*B*)	cp (x)	(*g*)	0
7	June 8	II	..	a, control	(*g*)	0
8	June 11	II	*B*	bm (y) 2.5[h]	85	24.7
9	do	II	*B*	bn (y) 2.5[h]	98	2
10	(June 11)	II	..	b2.5 (control)[h]	(*g*)	0

[a] The numbers given indicate in centimeters the length of the plumules in Nos. 1 and 3, and the average height of the plants in Nos. 8 and 9. In the latter case the plants were mostly unbranched as yet. In Nos. 10 and 11 the plant were younger than in No. 3.

[b] Kept outside in cloth bag through the winter at Amarillo.

[c] Planted apart from the rest to avoid contamination from inoculated rows.

[d] Inoculations by the author, assisted by Dr. N. E. Stevens.

[e] These numbers indicate the time, in days, between setting the seed to germinate and inoculating and planting it.

[f] Inoculations performed by Dr. E. M. Freeman and Mr. J. H. Parker.

[g] Plants not counted.

[h] This number indicates the approximate length of the plumules in centimeters at the time of inoculation and planting.

TABLE V.—*Results showing infection produced in Red Amber sorgo by extraseminal inoculations*—Continued

PLAT F [a]

[Planted at Washington, D. C., in the greenhouse in beds separated by partitions 1 foot deep in the soil; counted Apr. 3, 1913.]

Serial No.	Date.	Seed lot.	Spore lot.	Method.	Total number of plants.	Infection.
						Per cent.
1	Nov. 1–9	V	*B* and *C*	Various [b]	67	55.2
2	do	V	..	a, control	16	0
2 [c]	do	V	..	do	16	31.25

[a] Inoculations by the author.

[b] These inoculations were performed with various methods and stages of growth in an effort to get more exact information. With the small number of plants, necessitated by the use of a greenhouse, differences in the amount of infection appearing were of little significance in view of the impossibility of properly controlling conditions. Most of the plants were not directly watered, except at planting (nor were they, in the control), until mature in the spring. Although all were grown in separate beds instead of pots and obtained ample moisture from below, they were much stunted by greenhouse conditions.

[c] The same plants as above, but counted Oct. 3, 1913.

While most of the results of these inoculations are positive beyond a doubt, an important negative result, as yet unexplained, should be pointed out. The conidia, in spite of the care taken to be certain of their identity (see p. 341), have never produced the slightest evidence of infective power in the few trials made in the field (plat C, Nos. 1, 2, and 9; plat D, Nos. 2, 3, and 9; and plat E, Nos. 2 and 6). Brefeld (1895, p. 30) has found that oat smut, like many other pathogenic organisms, loses its virulence after several months in artificial cultures. Unless *Sorosporium reilianum*, as cultivated on carrot agar in these investigations, lost its infective power very quickly, however, this explanation does not seem adequate, for new cultures grown artificially for only two or three weeks produced no infection when inoculated on 15 plants at the same time and under the same conditions as plat F, No. 1. The conidia have not been observed to produce infection threads as figured by Brefeld (1883, pl. 11, fig. 7).

The first question which arises on considering the fact, here now clearly shown, that extraseminal infection does take place, is, What factor has been introduced to bring about successful infection when so many former attempts had failed? The results given in Table V, while not exhaustive, do make clear several of the essential points in the parasite's life history which will at least partially answer this question. The method designated under the abbreviation bmx will be observed to have produced the most consistently positive results wherever tried. Except at Amarillo, the only other methods which produced over 20 per cent infection were bn(x) in pots in the greenhouses of the Department (plat B, Nos. 1 and 2 of Table V), amx at St. Paul (plat E, No. 4), and bm(y) at St. Paul (plat E, No. 8), besides the inoculations later attempted in the greenhouse (plat F).

Since none of these methods can be presumed to correspond closely to the natural process of infection, the conclusions drawn from them must be largely a matter of inference. The small number of plants and the abnormal conditions in the greenhouse make it unnecessary to consider method bn(x), in plat B, Nos. 1 and 2, further than to note that both seeds and soil were heavily inoculated and that the seeds were germinating. Moreover, method bmx in the same series (plat B, Nos. 3 and 4) produced 100 per cent of infection on six plants, so that both these methods appear to have been proportionately more successful than elsewhere, probably because of the more thorough technique where so few plants were concerned. It appears, indeed, that the abundance of infectious material provided has been the most salient factor involved. Without it at Amarillo natural infections were often so numerous that the effect of inoculation was not perceptible; compare, for instance, plat A, No. 7, with plat A, Nos. 9 and 13, and plat A, No. 8, with plat A, Nos. 10 and 14. Method amx, which is closely similar to bmx on account of the large amount of spore material provided, the seedling having to grow up through the latter in both cases, has also produced a comparatively large percentage of infection, even exceeding bmx (plat E, No. 3) at St. Paul.

These results immediately suggest that no such crucial period for infection of the seedling obtains in the case of this smut as has been observed by Brefeld (1895, p. 46) for *Ustilago cruenta*, for the presence of the infecting organism during the whole of the early development of the host produces the disease when its presence on the seed alone will rarely do so. While *U. cruenta* was not able to infect, in Brefeld's experiments, after the leaf sheath had been split as far down as 1 cm. from the tip, the plumules of the plants inoculated by method bm(y)—a dusting of dry spores over the seedlings—in plat E, No. 8, averaged close to 2.5 cm. in length and yet were nearly as abundantly infected as those which were smaller and more heavily inoculated four days before (plat E, No. 3).

The difference between the latter and plat E, No. 4 (method amx) is not sufficient to militate against the conclusion that a late period of infection is possible, although it has seemed from the character of the infection in the mature plant, as revealed by the histological studies already discussed, that the infection in the field at Amarillo is usually quite early in its origin. That it is systemic in the individual culm more characteristically than in the plant as a whole, however, supports this idea of late infection (see p. 348). Investigation has shown, moreover, that the hyphæ were at least not widely disseminated in the growing tissues of several seedlings which later developed infection. In the seasons of 1910[1] and 1911 about 200 seedlings at three to four weeks after planting were dissected and a part of the meristem—that containing the primary

[1] The dissections in this season were made by Mr. V. L. Cory.

growing point—was removed and preserved. The plants were then induced to produce a second growth from what remained. The meristem of those which developed head smut at maturity was then carefully examined; yet in none of the 16 plants which developed the disease could the hyphæ be found in the parts preserved.

In additon to the negative evidence of these dissections, Mr. Karl F. Kellerman, of the Bureau of Plant Industry, stated to the writer in recent conversation that he performed a number of experiments with this smut by artificial inoculations on sorghum in the greenhouse while working in Ohio with his father, Dr. W. A. Kellerman. The plants were in pots and were inoculated at stages varying from the time they first appeared above ground until they were about 5 inches high. The method used was to wash the soil away from the roots, sift dry spores over them, and re-cover with soil. While some indications pointed to infection through the roots, this was not definitely established. Whatever the mode of entry, however, the parasite proved able under the conditions in the greenhouse to infect plants at all the stages at which they were inoculated.

In the recent greenhouse experiments (Table V, plat F, No. 1) some of the plants were successfully inoculated after the first leaf had begun to turn green. But, most unexpected of all, after leaving these plants to grow all summer it was found in October that the control (plat F, No. 2) contained five smutted plants, whereas the original culms which developed in April showed no sign of the disease. Other plants, too, which had not been smutted in the spring had grown smutted culms by fall. While Hecke (1907, p. 572) has presented similar facts as proof of shoot or branch ("Trieb") infection by *Ustilago antherarum*, in the case of sorghum, at least, there is some uncertainty as to the exact point of infection. The inoculation of the nodal buds has been tried a few times in the greenhouse without result. This does not preclude the possibility of such an infection, however, and more careful work supported by histological observations is needed.

It does not seem that the spread of the disease from plant to plant under greenhouse conditions makes it probable that such an occurrence is at all common in the field, but it does add certainty to the conclusion that infection by this smut is by no means confined to the early seedling stage of the host. This, then, together with the sparse germination of the spores, readily explains the repeated failures to produce any appreciable amount of infection by inoculation of the seed.

In Table V, plats C, D, and E, it will be observed that the same lot of seed, "Seed lot II," previously treated with a 0.24 per cent solution of formaldehyde, was used for nearly all the inoculations. This seed produced plants free from head smut at both Manhattan, Kans., and St. Paul, Minn. (plats D, Nos. 10, 11, 12, and 13, and E, Nos. 7 and 10), except when artificially inoculated; but at Amarillo all but one of the

control plantings (plat C, Nos. 12 to 20, inclusive) were infected—two of them to the extent of 25 per cent or more—while the percentage of infection in the successful inoculations was not remarkably greater, as compared with controls, than was produced by the same methods at the other two stations. It is thus indicated that at Amarillo, or wherever this smut occurs at all commonly, the parasite is present, doubtless in the soil, in much the same way as the common maize smut, *Ustilago zeae*, is present where maize is much grown.

PREVENTION OF HEAD SMUT

Since the period of infection appears to be quite indefinite, the prevention of this disease seems almost as difficult a problem as that of dealing with common maize smut, and, where prevalent, is a more serious question on account of the more systematic character of the infection. This latter fact, however, suggests a possible, though very doubtful and as yet untried, specific measure for prevention—i. e., the treatment of the soil about the seed at planting time in some such way as is done for onion smut—in the hope of keeping infection away from such buds as develop early in the life of the plant.

The fact that the disease occurs most abundantly in a district where manures or fertilizers have rarely, if ever, been used obviates the explanation of its occurrence on this basis. The Panhandle of Texas is, however, a region of high winds favorable to its spread, and the cutting out and burning of the whole plant when one is found infected should, of course, be recommended. Rotations planned to avoid continuous cropping of the particularly susceptible sorgo varieties on the same ground or to the leeward of prevailing winds from such a field should also considerably reduce the amount of head smut.

An important element in the relation of the problem to the grain-sorghum grower is the fact that milo, as has been noted by Freeman and Umberger (1908), is a variety apparently immune from all the sorghum smuts. This crop is widely grown in the southern part of the Great Plains, and it should be possible, theoretically, to obtain various immune varieties adapted to other sections by breeding from it. Since the cause of this immunity is not yet apparent, however, it can not be definitely stated that its hybrids will partake of this character. Kafir and broom corn, while much less susceptible to this smut than the sorgos, are quite subject to the attack of the kernel smut. This lack of immunity might prove serious to these crops or even to maize, should the head smut ever become as abundant as has maize smut (*Ustilago zeae*) in many sections. The latter is indigenous to America, however, and since the head smut is not, it may be hoped that adequate quarantine measures would prevent its spread and lead, perhaps, to its final eradication.

SUMMARY

(1) The head smut of sorghum, *Sorosporium reilianum* (Kühn) McAlpine, was first reported from Egypt in 1868. It has been found to be a destructive parasite, though not yet of widespread occurrence in this country. It occurs also on maize, or Indian corn.

(2) The organism has been grown in artificial culture. Its growth is almost exclusively conidial under favorable conditions, the optimum temperature being 28° to 30° C. As with several others of the Ustilagineae, spore-like bodies are occasionally found in older cultures.

(3) Although perfect sori of the parasite are not usually produced in every head of a plant, most of the stools and branches are so affected, even when producing no spore-bearing tissue, that the inflorescence is sterile and often peculiarly proliferated. This vegetative stimulus results also in the development of the lateral buds into branches.

(4) Histological studies indicate an early period of infection and the systemic nature of the disease. The lateral buds carry the infection in their meristematic tissue apparently from the time of their formation when the culm is starting to differentiate the nodes.

(5) The work of other investigators, though not conclusive, pointed to infection from seed-borne spores and the possibility of applying the usual seed-treatment methods for preventing the disease. Both of these contentions have been shown to be untenable by an extensive series of ecological experiments and exhaustive tests of various sterilizing agents, including the use of thermal methods, on the seed.

(6) Numerous floral inoculations failed to show that the infection was produced intraseminally and carried over in the seed to the next crop. On the other hand large percentages of infection were repeatedly produced by inoculation of the seedlings with dry spore material, some becoming infected in the greenhouse even after the first leaf had emerged from the sheath and begun to turn green. While the process of infection has not yet been observed histologically, it is clearly proved that the parasite is not carried with the seed, but is wind-distributed in the locality in which it occurs, doubtless infecting the seedling from the soil.

(7) Though widely distributed in the tropical and semitropical countries of the world, the head smut has been known in this country for only about 35 years. Methods of combating it are especially needed in order to prevent its spread. Fortunately the widely grown variety, milo, has proved immune from all the smuts of sorghum.

LITERATURE CITED

APPEL, OTTO, and RIEHM, E.
1911. Die Bekämpfung des Flugbrandes von Weizen und Gerste. *In* Arb. K. Biol. Anst. Land- u. Forstw., Bd. 8, Heft 3, p. 343–426, 2 fig., 1 pl.

BARBER, C. A.
1904. Diseases of Andropogon sorghum in the Madras presidency. *In* Dept. Land Records and Agr. Madras, Agr. Branch Bul., v. 2, no. 49, p. 276–277.

BREFELD, OSCAR.
1883. Die Brandpilze. I. 220 p., 13 pl. Leipzig. (*His* Untersuchungen aus dem Gesammtgebiete der Mykologie. Heft 5.)
1895. Die Brandpilze. II–III. 236 p., 12 pl. Münster i. W. (*His* Untersuchungen aus dem Gesammtgebiete der Mykologie. Hefte 11–12.)

BUSSE, WALTER.
1904. Untersuchungen über die Krankheiten der Sorghum-Hirse. *In* Arb. K. Gsndhtsamt. Biol. Abt., Bd. 4, Heft 4, p. 319–426, 12 fig., pl. 5–6.

CLINTON, G. P.
1897. Broom-corn smut. Ill. Agr. Exp. Sta. Bul. 47, p. 375–412, illus.
1900. Head smut of sorghum. Cintractia reiliana (Kühn). Ill. Agr. Exp. Sta. Bul. 57, p. 346–347.
1904. North American Ustilagineæ. *In* Proc. Boston Soc. Nat. Hist., v. 31, no. 9, p. 329–529.

COOKE, M. C.
1876. Some Indian fungi. *In* Grevillea, v. 4, no. 31, p. 114–118, pl. 63.

CUGINI, GINO.
1891. Il carbone del grano turco. *In* Bol. R. Staz. Agr., Modena, n. s., v. 10, 1890, p. 71–87, pl. 3–4.

DANDENO, J. B.
1908. On the toxic action of bordeaux mixture and of certain solutions on spores of fungi. *In* 10th Rpt. Mich. Acad. Sci., p. 58–62.

DIETEL, PAUL.
1900. Hemibasidii (Ustilagineae und Tilletiineae). *In* Engler, Adolf, and Prantl, K. A. E. Die Natürlichen Pflanzenfamilien. T. 1. Abt. 1**, p. 1–24, 13 fig. Leipzig.

EVANS, I. B. POLE.
1911. Maize smut or "Brand." *Sorosporium reilianum* (Kühn) McAlp. *In* Agr. Jour. Union S. Africa, v. 1, no. 5, p. 697, 1 pl.

FAILYER, G. H., and WILLARD, J. T.
1890. Experiments with sorghum. Kans. Agr. Exp. Sta. Bul. 16, p. 135–149.

FREEMAN, E. M., and JOHNSON, E. C.
1909. The loose smuts of barley and wheat. U. S. Dept. Agr. Bur. Plant Indus. Bul. 152, 48 p.

——— and UMBERGER, H. J. C.
1908. The smuts of sorghum. U. S. Dept. Agr. Bur. Plant Indus. Circ. 8, 9 p. Rev. ed., 8 p. 1910.

GRÜSS, J.
1902. Biologische Erscheinungen bei der Cultivirung von Ustilago maydis. *In* Ber. Deut. Bot. Gesell., Jahrg. 20, Heft 4, p. 212–220, pl. 11.

HACKEL, EDUARD.

1887. Gramineae (echte Gräser). *In* Engler, Adolf, and Prantl, K. A. E. Die Natürlichen Pflanzenfamilien. T. 2, Abt. 2, p. 1–97, 108 fig. Leipzig. For English edition, see The True Grasses. Tr. by F. Lamson-Scribner and Effie A. Southworth. 228 p., 110 fig. New York, 1890. Reprinted, Westminster, 1896.

HAWKINS, L. A.

1913. The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. *In* Physiol. Researches, v. 1, no. 2, p. 57–92, 6 fig.

HECKE, L.

1907. Die Triebinfektion bei Brandpilzen. *In* Ztschr. Landw. Versuchsw. Oesterr., Jahrg. 10, Heft 6, p. 572–574.

HENNINGS, P. C.

1896. Pilzkrankheiten des Sorghums (Andropogon sorghum (L.) Brot.). *In* Notizbl. K. Bot. Gartens u. Mus. Berlin, Bd. 1, No. 4, p. 118–121.

HERZBERG, PAUL.

1895. Vergleichende Untersuchungen über Landwirtschaftlich Wichtige Flugbrandarten. 33 p. Halle. (Inaugural Dissertation.) *Reprinted in* Zopf, W. F. Beiträge zur Physiologie und Morphologie niederer Organismen. Heft 5, p. 1–36, 3 pl. Leipzig.

HITCHCOCK, A. S., and NORTON, J. B. S.

1896. Corn smut. Kans. Agr. Exp. Sta. Bul. 62, p. 169–212, 10 pl.

HORI, SEITARO.

1907. Seed infection by smut fungi of cereals. *In* Bul. Imp. Cent. Agr. Exp. Sta. Japan, [Nishigahara, Tokio], v. 1, no. 2, p. 163–176.

JENSEN, J. L.

1888. Om Kornsortemes Brand. Medd. 2, 72 p. Kjøbenhavn.

JOHNSTON, T. H.

1910. Maize-smut. *In* Rpt. Govt. Bur. Microbiol. [N. S. Wales], 1909, p. 70, 1 fig.

1910a. Maize-smut. *In* Agr. Gaz. N. S. Wales, v. 21, pt. 1, p. 43–44, 2 fig.

KELLERMAN, W. A.

1891. Experiments with sorghum smuts. Kans. Agr. Exp. Sta. Bul. 23, p. 95–101.

1898. Ustilago reiliana. (Abstract.) *In* 6th Ann. Rpt. Ohio State Acad. Sci., [1897], p. 40.

1900. Experiments with the sorghum smuts. *In* 8th Ann. Rpt. Ohio State Acad. Sci., [1899], p. 18–19.

1900a. A foliicolous form of sorghum smut and notes on infection experiments. *In* Ohio Nat., v. 1, no. 1, p. 9–10, pl. 2.

——— and JENNINGS, O. E.

1902. Smut infection experiments. *In* Ohio Nat., v. 2, no. 6, p. 258–261, 1 fig.

——— and KELLERMAN, K. F.

1899. Studies of Ustilago reiliana. *In* 7th Ann. Rpt. Ohio State Acad. Sci., [1898], p. 38.

——— and SWINGLE, W. T.

1890. Notes on sorghum smuts. *In* Trans. 23d Meeting, Kans. Acad. Sci., v. 12, [pt. 2], p. 158–159, 1 pl.

KÜHN, JULIUS.
1875. Ustilago reiliana. *In* Rabenhorst, G. L. Fungi Europaei Exsiccati . . . ed. nova, s. 2, cent. 20, no. 1998. Dresdae.
1878. Die Brandformen der Sorghumarten. *In* Hedwigia, Bd. 17, No. 1, p. 6–14.

KUSANO, SHUNSUKE.
1911. On the chloranthy of Prunus mume caused by Caeoma makinoi. *In* Jour. Col. Agr. Imp. Univ. Tokyo, v. 2, no. 6, p. 287–326, pl. 17–18.

MCALPINE, DANIEL.
1910. The smut of maize and its treatment. *In* Jour. Dept. Agr. Victoria, v. 8, pt. 5, p. 290–298, 11 fig.
1910a. The Smuts of Australia. 288 p., 15 fig., 56 pl. Melbourne.

MOORE, G. T., and KELLERMAN, K. F.
1904. Method of destroying or preventing the growth of algæ and certain pathogenic bacteria in water supplies. U. S. Dept. Agr. Bur. Plant Indus. Bul. 64, 44 p.

MOTTARELE, GIOVANNI.
1903. L'Ustilago reiliana f. zeae e la formazione dei tumori staminali nel granone. *In* Ann. R. Scuola Sup. Agr. Portici, s. 2, v. 4, 17 p.

MUNDY, H. G.
1910. Maize smut. [Rhodesia] Dept. Agr. Bul. 46, 4 p., 2 fig.

MUNERATI, OTTAVIO.
1910. La Sphacelotheca reiliana (Kühn) nel Sorghum halepense Pers. *In* Staz. Sper. Agr. Ital., v. 43, fasc. 10, p. 718–722, 2 fig.

NORTON, J. B. S.
1895. Ustilago reiliana on corn. *In* Bot. Gaz., v. 20, no. 10, p. 463.
1896. A study of the Kansas Ustilagineae, especially with regard to their germination. *In* Trans. Acad. Sci. St. Louis, v. 7, no. 10, p. 229–241, pl. 25–29.

PASSERINI, GIOVANNI.
1876. Ustilago reiliana J. Kühn. *In* Rabenhorst, G. L. Fungi Europaei Exsiccati . . . ed. nova, s. 2, cent. 1 (resp. cent. 21), no. 2096.
1877. Funghi Parmensi. Fam. IV.–Ustilaginei. *In* Nuovo Gior. Bot. Ital., v. 9, no. 3, p. 235–239.

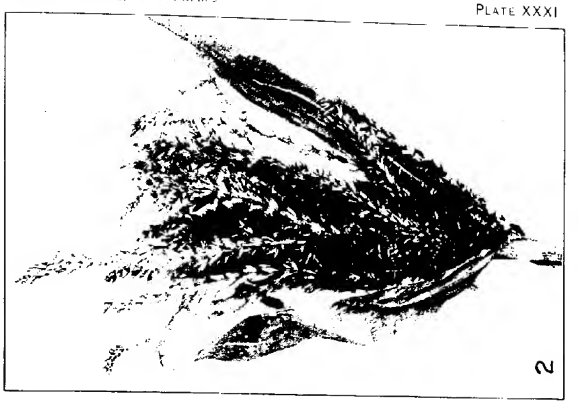
POTTER, A. A.
1912. Ustilagineae on Andropogon sorghum (L.) in America. (Abstract.) *In* Phytopathology, v. 2, no. 2, p. 98.

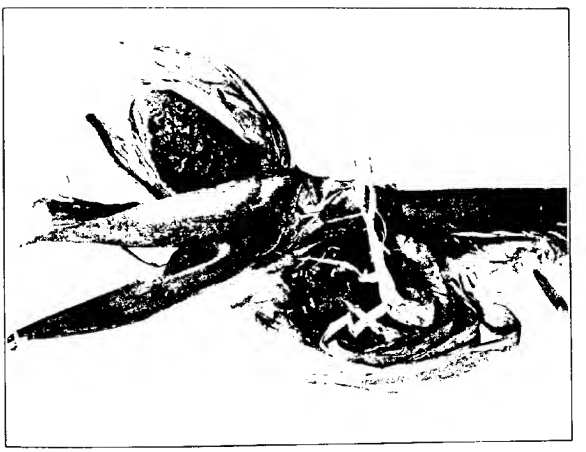
SACCARDO, P. A.
1876. Ustilago reiliana. *In* Rabenhorst, G. L. Fungi Europaei Exsiccati . . . ed. nova, s. 2, cent. 1 (resp. cent. 21), no. 2095.

TONI, G. B. DE.
1888. Sylloge Ustilaginearum et Uredinearum. *In* Saccardo, P. A. Sylloge Fungorum. v. 7, p. 471. Patavium.

PLATE XXXI

Fig. 1.—Head smut in ear of maize (after McAlpine).
Fig. 2.—Head smut in tassel of maize (after Evans).


2




a
b
c
1


2

PLATE XXXII

Fig. 1.—The three American species of sorghum smut on Blackhull kafir: (a) *Sphacelotheca cruenta*, (b) *Sorosporium reilianum*, (c) *Sphacelotheca sorghi*. Photographed by author.

Fig. 2.—Head smut, *Sorosporium reilianum* (Kühn) McAlp., on "sumac" sorgo, San Antonio, Tex., October, 1913. Photographed by Mr. Karl F. Kellerman.

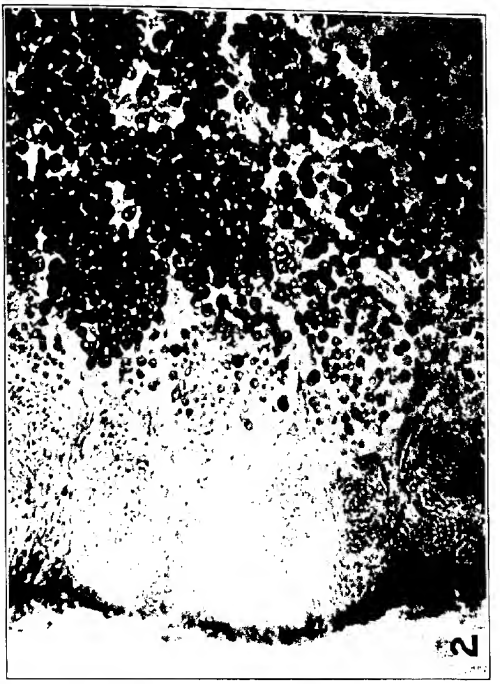
51131°—14——4

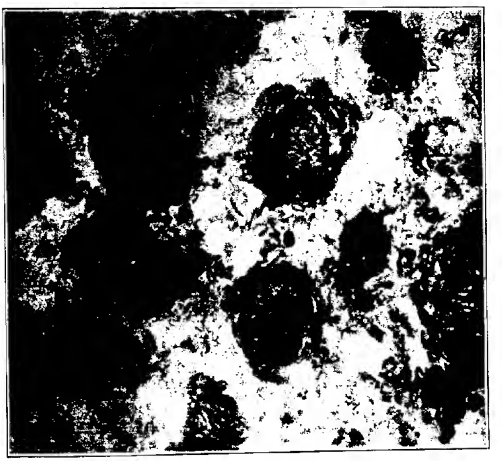
PLATE XXXIII

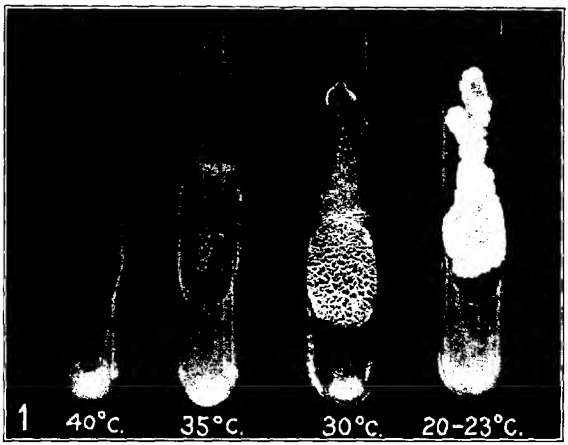
Fig. 1.—Section through young sorus, showing hyphal aggregates preceding spore formation. × 710. Photomicrographed by author.

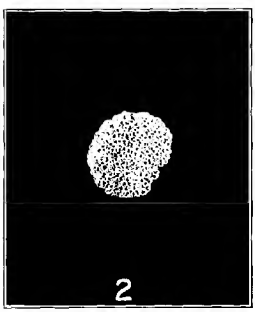
Fig. 2.—Section through immature sorus. Note the fibrovascular bundle on the left, about which the spores, none of which were as yet quite mature, were developing in groups even in the earliest stages. × 365. Photomicrographed by author.

PLATE XXXIII






1
40°C.
35°C.
30°C.
20-23°C.


2

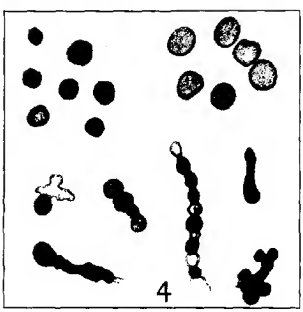

4

PLATE XXXIV

Fig. 1.—Growth of organism of head-smut of sorghum and maize on carrot agar at various temperatures; cultures about 6 weeks old. × 4/5.

Figs. 2 and 3.—Twenty-two days' growth of organism of head-smut of sorghum and maize on synthetic glucose agar (fig. 2) and on carrot agar (fig. 3). Photographed by Mr. E. C. Johnson and author.

Fig. 4.—Chlamydospores of organism of head-smut of sorghum and maize formed in culture in peptonized maltose solution. In the upper right-hand corner are shown some natural spores for comparison. × 430. Drawn by author.

PLATE XXXV

Fig. 1.—Smutted culms of Amber sorgo, showing the characteristic sterility of the main panicle. Photographed by Mr. E. C. Johnson.

Fig. 2.—Proliferated head of Blackhull kaoliang, with one normal and one smutted head. Photographed by author.

Fig. 3.—Smutted and nonsmutted plants of Red Amber sorgo used in head-smut infection experiment. Control pot (see Table V, plat B, No. 8) on left; inoculated pot (Table V, plat B, No. 3) on right, showing three smutted plants. Photographed by Mr. E. C. Johnson.


1
2
3

PLATE XXXVI

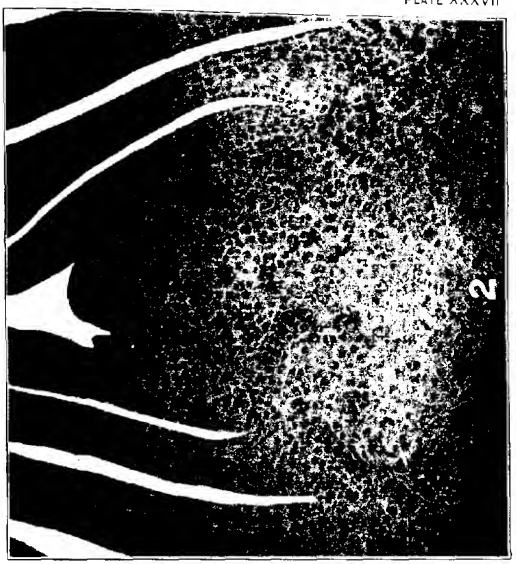
Panicular formation in apex of proliferated sorghum flower. Longitudinal section, showing presence of hyphæ of head smut. × 70. Photomicrographed by Mr. W. W. Gilbert and author.

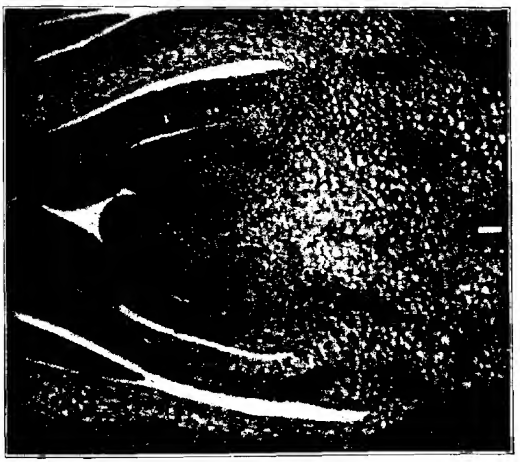
PLATE XXXVII

Longitudinal sections through the growing points of two of the buds indicated in text figure 1, showing hyphæ of the head smut. × 150.

Fig. 1.—Bud 3 of culm B1. Positions of hyphæ are shown in text figure 2.

Fig. 2. Bud 7 of culm N2. Photomicrographed by Mr. W. W. Gilbert and author.


2


1

OXIDASES IN HEALTHY AND IN CURLY-DWARF POTATOES

By H. H. BUNZEL,

Chemical Biologist, Plant Physiological and Fermentation Investigations, Bureau of Plant Industry

INTRODUCTION

The curly-dwarf, among other related potato diseases, has been described very fully in one of the recent publications of this Department (Orton, 1914),[1] in which the very confusing literature on the subject of potato maladies referred to vaguely in the past as leaf-roll, curly-top, blight, Kräuselkrankheit, Blattrollkrankheit, etc., is critically reviewed. On the basis of this review and of the work done by the Office of Cotton and Truck Diseases and Sugar-Beet Investigations, which has made a thorough survey of the principal potato districts on this continent, as well as abroad, a number of distinct diseases are recognized, each with its characteristic symptoms and probable cause.

Some of these diseases, particularly the leaf-roll and the curly-dwarf, can not be traced to organisms of any sort for their origin and are supposedly disturbances of a purely physiological nature. To throw light on this matter, Mr. W. A. Orton, of the Bureau of Plant Industry, requested the writer to make a quantitative study of the oxidizing enzyms of potatoes at Houlton, Me., and immediate vicinity. Oxidase determinations were there carried out with healthy material, as well as with plants having the curly-dwarf disease. In this paper only such plants were considered to have curly-dwarf as showed the characteristic symptoms described by Orton (1914).

This is not the first attempt to correlate enzymatic disturbances with plant diseases. Sorauer (1908) was the first one to attribute the leaf-roll of potatoes to disturbances in the oxidase mechanism of the tubers. According to this author, the dark patches observable on the cut surfaces of such tubers are due to a greater oxidase content than is found in normal tubers; the abnormalities of the foliage are due to malnutrition through the tubers. His conclusions are based on chemical experiments of Grüss (1907). The most important and complete investigation of the subject was made by Doby (1911–12), who reached the very important conclusion that the oxidase content of the diseased tubers is greater than that of the normal ones. He also found a higher ash content and lower percentage of starch and insoluble protein in the diseased tubers, stating

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 403-404.

Journal of Agricultural Research,
Dept. of Agriculture, Washington, D. C.

Vol. II, No. 5
Aug. 25, 1914
G—30

that the increased ash is probably responsible for the increased oxidase activity, judging from the work of Bertrand (1897) and others (Dony-Hénault, 1908; Dony-Hénault and Van Duuren, 1907; Trillat, 1903 and 1904). The diminution of the starch and protein in the diseased tubers is the net result of the increased combustion of their cleavage products. Doby (1911–12) measured the oxidation of pyrogallol according to the method of Bach and Chodat (1904), with and without the addition of hydrogen peroxid, and also measured the oxidation of tryosin according to an optical method (König and Krüss, 1904). In general, the peroxidase, oxygenase, and tyrosinase were present in larger quantities in the curly-dwarf tubers than in the normal ones.

With the manometric method devised by the writer, it was found that in sugar beets affected with curly-top the oxidase content of the foliage is two to three times as great as that of normal beet leaves. This same difference was found to exist when plants were studied whose growth had been retarded by causes other than the curly-top (1912).

DESCRIPTION OF EXPERIMENTAL METHODS

For all of the experiments with leaves, fresh material was used—that is, plants collected on the day of experimentation. In the case of tubers it was sometimes necessary to allow them to lie for a few days until enough material for a complete series of experiments had accumulated. During the whole period of 10 weeks the plants were collected in the field at 7.15 a. m. and taken to the laboratory at once. The weight of the foliage was there determined and the material then ground up in a meat chopper. The juice was pressed out of the pulp by hand through a silk cloth.

Nearly all of the experiments with normal material were made on plants of one variety, the Green Mountain. With one or two intentional exceptions the samples of normal plants were taken from the same field. They were grown under fairly uniform conditions of environment, and the soil was fertilized uniformly with the same fertilizer. All of the pathological material was collected on a field several miles away, necessarily from plants of different varieties, but all grown on the same type of soil and with the same kind of fertilizer as that used in the field on which the normal plants were collected. In most cases 25-gram samples of the juice were preserved with about 100 c. c. of 25 per cent alcohol, in order that the solid contents of the juices examined might be determined, in case it seemed necessary.

The experiments were carried out in the same manner as that described in a former publication (Bunzel, 1912). The following 18 ring compounds were used as reagents to determine the oxidase activity of the juices: Benzidin, pyrogallol, alphanaphthol, leuco base of malachite green, phloroglucin, aloin, pyrocatechol, tyrosin, hydrochinone, phloridzin,

resorcin, guaiacol, orthocresol, metacresol, paracresol, orthotoluidin, metatoluidin, and paratoluidin.

In the case of each of the solid substances 0.05 gram was weighed out for each determination In the experiments with guaiacol 4 drops (0.15 gram) were used; by separate experiments it was shown that this quantity gave the highest result under the conditions of the experiments. The cresols and toluidins were found to be very poisonous, inhibiting the action of the potato oxidases when used in too great quantities. By a series of experiments it was found that 2 drops of each gave the optimum result. As in previous experiments, 1 c. c. of normal sodium hydrate was used in the glass basket in all experiments with pyrogallol, to absorb the carbon dioxid produced during the oxidation.

All of the experiments described herein were carried out at 41° C. The apparatus used were all of the small type, in which a change in pressure of 1 cm. of mercury corresponds to the absorption of 1 c. c. of oxygen. The rate of shaking was 5 complete excursions in 3 seconds. All of the results were expressed in terms of the oxidase unit previously used by the writer. The unit is an oxidase solution of such strength that 1 liter of it can bring about the oxidation of the equivalent of 1 gram of hydrogen (1912, p. 40). Blank determinations with the reagents here used showed that no measurable oxidase absorption took place under the conditions of the experiments in the absence of plant juice.

The thermostat box was provided with a false bottom about 6 inches above the floor of the box and a free space of 4 inches at each end for the sake of free circulation. The heating lamps were all arranged below this false bottom. In this way very uniform heating throughout was attained. The stopcocks were closed through an opening just large enough to admit the arm, instead of opening a window, as was done formerly. To reduce still more the disturbances of temperature within the box, the opening for the arm was protected by means of a sleeve into which the arm was slipped.

Although the results obtained with the method here used are more accurate and reliable than those obtained with any other existing method, yet this method is not entirely free from sources of error. It is probable that a part of the oxidases are destroyed by the shaking at the comparatively high temperature (41° C.). It is also probable that the reagents used for the oxidation act as poisons even in the small concentrations in which they are present. Probably, however, it will be only a matter of time before these possible sources of errors will be eliminated. For the present it may be said that the results were obtained in experiments carried out under identical conditions and are therefore comparable. In all the experiments the juice was pressed out of the ground pulp by hand and by the same operator. While it may seem that juices of more uniform composition might be obtained by pressing them out with a

machine, separate experiments show that a portion of the activity is lost thereby. The fresh hand-pressed potato juice had an activity of 0.287 units (pyrogallol), while the juice pressed out of the remaining pulp by means of a hydraulic press at a pressure up to 15 tons on a 6-inch circular ram was 0.170 units (pyrogallol) and the juice pressed out at a still higher pressure had an activity of only 0.107 units (pyrogallol). Inasmuch as the juice obtained by means of the hydraulic press had to pass through an appreciable amount of compressed pulp, it is probable that the diminished activity of the machine-pressed juice was due to the loss of a part of the oxidases by adsorption.

In this connection the results of Dixon and Atkins (1913) are very interesting. They found that in successive pressings of leaves (*Hedera helix*) in a vise, juices with increasing concentration of electrolytes were obtained. They experimented also with leaves treated with liquid air and concluded that the only way to obtain juices corresponding to the concentration of the sap in the vacuoles of the uninjured tissues is to press them out after exposure to liquid air. Unfortunately, such procedure was impossible during this work, which had to be carried out in the field.

RATE OF GROWTH OF THE POTATO PLANT

In former publications it has been shown that the oxidase content of juices bears a very definite relation to the rate of development of the particular plant specimens from which they are derived. In the sugar beet, which the writer studied in this respect, the oxidase content of the foliage runs up appreciably when the normal growth of the plants is interfered with by drought, excessive watering, diseases, etc. In the foliage of normally developing sugar-beet plants the oxidase content of the juice is only about one-half that of stunted plants. On the basis of the results obtained with sugar beets the following generalizations can be made:

Normal growth.....Normal (low) oxidase content.
Retarded growth.....Abnormal (high) oxidase content.

The recognition of this fact led to an examination of the rate of growth of the potato plants which were used in this research. Table I shows the relation which the size of the shoots and the foliage of all the plants in a hill, as well as of the single shoots, bears to the age of the plants.

TABLE I.—*Relation of the total weight of the shoots of the whole hills, as well as of the single shoots, to the age of the potato plants*

Series No.	Date of collection.	Age.	Total weight of shoots.	Number of hills.	Mean total weight of shoots per hill.	Number of shoots.	Mean weight of shoots.
		Days.	*Grams.*		*Grams.*		*Grams.*
1	July 9	29	84	5	17		
2	July 10	30	102	7	15	17	6
4	July 11	31	150	8	19	22	6.8
6	July 12	32	108	8	13.5	19	5.7
9	July 14	34	222	15	15	32	6.9
10	July 15	35	297	9	33	23	12.9
12	July 31	19	75	7	11	40	1.9
14	July 31	19	100	6	17	41	2.4
15	Aug. 1	20	170	6	28	63	2.7
18	Aug. 2	60	360	1	360	3	120
21	Aug. 4	62	750	2	375	4	188
24	Aug. 8	66	680	1	680	4	170
26	Aug. 9	67	368	1	368	1	368
29	Aug. 11	30	365	5	73	44	8.3
32	Aug. 21	40	500	1	500	7	71.4
35	Aug. 29	88	350	1	350	1	350
38	Sept. 8	98	375	1	375	1	375

In order to present these data more clearly, they were plotted as shown in figure 1. The ages of the plants are measured off on the abscissæ and the weight of the shoots on the ordinates. The continuous line corresponds to the development of the plants (the total weight of the shoots of one hill), and the broken line corresponds to the mean rate of development of all of the single shoots of one hill.

The irregularities of the curve representing the growth of the shoots of a whole hill are apparently due to variations in the number of stalks contained therein. This becomes strikingly apparent from the smoothness of the curve representing the growth of single stalks. With practically no interruption this curve shows a gradual increase in size until the sixty-seventh day is reached, growth of the stalks apparently stopping at that point. The curve from this point on is practically a straight line.

OXIDASES OF HEALTHY POTATO PLANTS

In order to be able to compare the oxidase activities of diseased potato plants with healthy ones at the same stage of development, it was essential to establish the oxidase content of healthy material at all stages of development. Such a study on normal plants is also of general physiological interest. While the excellent work of Palladin (1906) and his school has shown that the respiration of plants takes place in stages corresponding to several distinct respiratory enzyms, they have made no measurements of the oxidizing power of these respiratory enzyms. Moreover, working with frozen wheat seedlings and those not frozen and with etiolated leaves of *Vicia faba* and leaves of *Plectogyne japonica*, they con-

clude that "oxidase" and "oxygenase" are practically lacking in embryonic organs and that their concentration in these plants rises during growth and diminishes again when growth has stopped. They draw their conclusions entirely from the quantity of CO_2 liberated by the

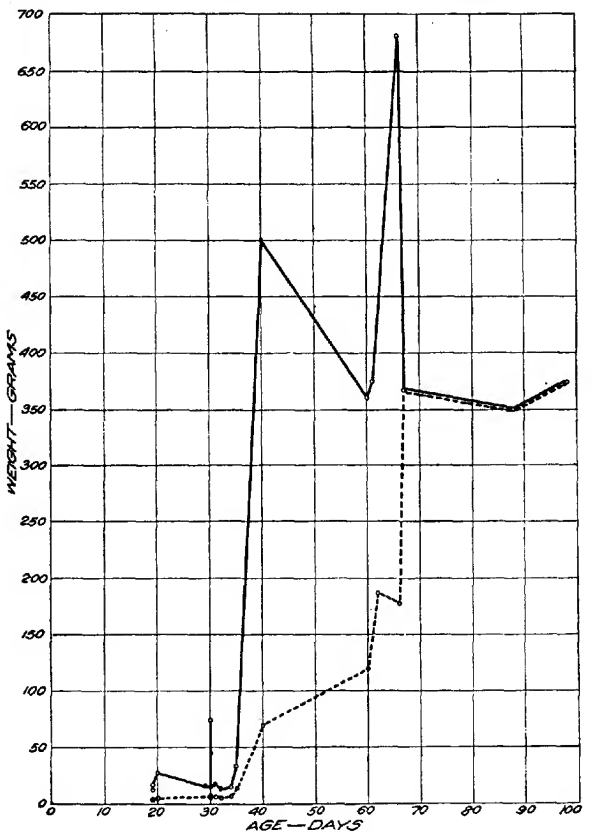


FIG. 1.—Rate of development of the aerial portion of potato plants and mean rate of development of all the single shoots in one hill.

plant organs under different conditions. It seemed of great interest, therefore, to find out what relation the concentrations of the oxidases present in the pressed-out sap of a plant bear to the state of development of the same plant.

OXIDASE ACTIVITY OF THE JUICE OF THE SHOOTS

The results obtained in the measurement of the oxidases in the juice of healthy potato plants of the same variety at various ages, grown under normal and as nearly identical conditions as possible, are given in Tables II to VIII, and some of the results are also shown graphically in figures 2 to 20.

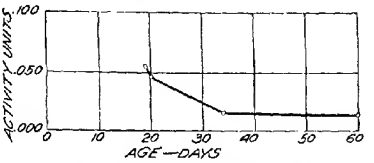


FIG. 2.—Curve showing oxidation of leuco base of malachite green in the presence of the juice of green potato shoots.

The shoots were taken from the plants immediately above the point where they emerged from the soil. In figures 2 to 5 [1] the abscissæ represent the age of the plants as measured from the time of planting, and the ordinates the activities of the juices as measured in the oxidation of the various aromatic compounds used. These data show a distinct downward tendency; there is apparently a marked diminution in the oxidase activities of the pressed-out juice of the shoots during the beginning of their growth.

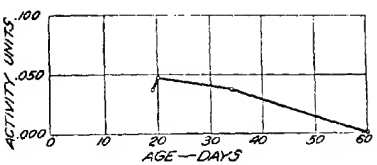


FIG. 3.—Curve showing oxidation of aloin in the presence of the juice of green potato shoots.

OXIDASE ACTIVITY OF THE JUICE OF THE STEMS

Experiments on sugar beets showed that the juice obtained from the stems of the plants exhibited very much less oxidase activity than that of the leaves (Bunzel, 1913a; 1913b). It seemed probable, therefore, that the juice of the stems of the potato plants examined would also be less active than the juice of the leaves. A comparative increase

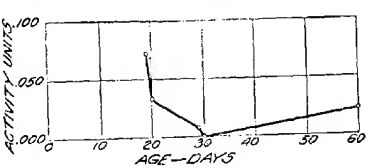


FIG. 4.—Curve showing oxidation of tyrosin in the presence of the juice of green potato shoots.

[1] Inasmuch as nearly all of the curves obtained in working with the 18 reagents show the same general relationships, for the sake of a briefer and, therefore, more comprehensive presentation of the facts, only 4 to 8 curves are presented in the case of the shoots, foliage, and tubers, respectively. While these curves were not picked at random, they are typical of the situation in each case. The writer felt justified in doing this, inasmuch as all of the curves can be constructed from the tables in the text, and since the results are numerically compared in Table XII.

TABLE II.—*Oxidase activities of the juice of shoots of healthy potato plants*

Series No.	Date.	Number of hills used.	Total weight of shoots.	Mean total weight of shoots per hill.	Date of planting.	Age of plant.	Oxidase activity expressed in units as measured in the oxidation of the reagents.																				
							Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	O-cresol.	M-cresol.	P-cresol.	O-toluidin.	M-toluidin.	P-toluidin.
			Grams.	*Grams.*		*Days.*																		
1	July 9	5	84	17	June 10	29								0.008	0.076									
2	July 10	7	102	15	June 10	30							0.047	0	.320									
3							0								.296			0.117						
4	July 11	8	150	19	June 10	31			0		0.008					0.179								
5																				0.281				
6	July 12	8	108	13.5	June 10	32													0.039					
7																					0.523			
8																			.105	.382	.507			
9	July 14	15	222	15	June 10	34				0.016		0.019					0							
10	July 15	9	297	33	June 10	35																0.062	0.047	0.039
11													.011		.476									
12	July 31	7	75	11	July 12	19							.023	.074	.553	.293	.078	.129						
13							.055	0	.027	.055	.047	.019												
14	July 31	6	100	17	July 12	19													.086	.312	.491			
15	Aug. 1	6	170	28	July 12	20	.074	.016	.016	.047	.047	.017												
16													.062	.031	.534	.215	.047	.086						
17																			.047	.218	.406	0	.047	.023
18	Aug. 2	1	360	360	June 3	60							.023	.023	.226	.086	.031	.020						
19							.016	0	.016	.012	.020	0												
20																			.043	.078	.152	.023	.039	.023

[a] Leuco base of malachite green.

in size of the stem of a growing plant as compared with the foliage or a diminution with age of the activity of the stem juice as compared with the activity of the foliage juice would therefore result in a diminution with age of the activity of the juice of the aboveground portion of the plant. Consequently oxidase determinations were made on the juice obtained from stems of plants 69 days old. The results are given in Table III.

TABLE III.—*Oxidase activities of the juice of stems of plants 69 days old*

Reagent.	Activity.[a]	Reagent.	Activity.[a]
Benzidin	0.035	Phloridzin	0.078
Pyrogallol	0	Resorcin	0
α-naphthol	0	Guaiacol	.023
Leuco base of malachite green	0	O-cresol	0
Aloin	.031	M-cresol	.101
Phloroglucin	0	P-cresol	.168
Pyrocatechol	.023	O-toluidin	0
Tyrosin	0	M toluidin	0
Hydrochinone	0	P-toluidin	0

[a] Activity expressed in units as measured in the oxidation of the reagents.

The stem juice proved to have no activity whatever toward 11 of these 18 reagents, and toward the remaining 7 it was slight in comparison with the activity of the foliage juice, as will be shown later. That the stem during growth increased in weight more rapidly than the remainder of the shoot is shown in Table IV (column 9). It is probable, therefore, that the diminishing activity of the juice of the shoots of the potato plant was due to increasing dilution with age of the very active leaf juice with the relatively inactive stem juice.

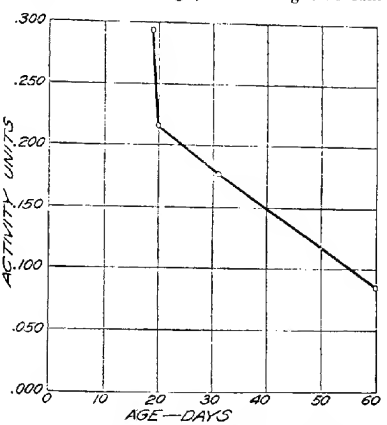


FIG. 5.—Curve showing oxidation of phloridzin in the presence of the juice of green potato shoots.

OXIDASE ACTIVITY OF THE JUICE OF THE LEAVES

The leaves proper are the seat of the greatest physiological activity in plants. The food of the plant is largely synthesized in the leaves and also in part broken down in them, according to the needs of the plant.

In physiological disturbances, such as the curly-dwarf disease of potatoes and the curly-top of sugar beets appear to be, the leaves are the parts primarily affected. It is therefore to be expected that any chemical differences existing between healthy plants and plants affected with the curly-dwarf disease will be most pronounced in the leaves proper. In order to be certain of results which represent the activity of the juice of

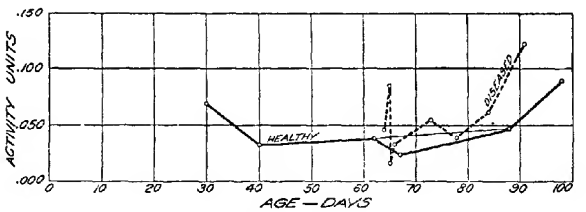


FIG. 6.—Curve showing oxidation of leuco base of malachite green in the presence of the juice of potato foliage.

the foliage proper, all experiments with the green parts of the potato plant were from this point on carried out on leaves alone. Table IV gives the data on the activity of the leaf juice alone.

Some of these results are also graphically presented in figures 6 to 12. (See footnote, p. 379.) For easy comparison the figures obtained with healthy leaves, as well as those obtained later with curly-dwarf leaves, were plotted on the same systems of coordinates. The continuous lines

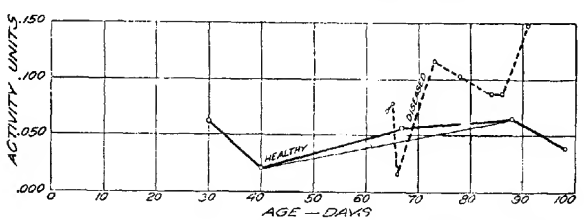


FIG. 7.—Curve showing oxidation of pyrocatechol in the presence of the juice of potato foliage.

represent the results obtained with healthy plants, the dotted lines those with the diseased ones.

These curves show great fluctuations of oxidase content. Barring some of the irregularities, probably due to individual peculiarities of the samples examined, the curves take a downward direction at first, remain at a low level for a prolonged period, and take an upward movement again towards the end. The lowest point in the curve is reached generally on the fortieth day; the period of low oxidase content extends to a point of time between the sixtieth and eightieth day, when the upward movement of

TABLE IV.—*Oxidase activities of the juice of the leaves of healthy potato plants*

Series No.	Date.	Number of hills used.	Total weight of shoots.	Mean total weight of shoots per hill.	Date of planting.	Age of plant.	Total weight of stems.	Stems × 100. Total weight of shoots.	Oxidase activity expressed in units as measured in the oxidation of the reagents. Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	O-cresol.	M-cresol.	P-cresol.	O-toluidin.	M-toluidin.	P-toluidin.
			Grams.	*Grams.*		*Days.*	*Grams.*	*Grams.*																		
21									....	....	....	....	....	....	....	0.043	0.456	0.109	0.059	0.059	....	....	....	....	....	....
22	Aug. 4	2	750	375	June 3	62	402	54	0.055	0.010	0.035	0.039	0.035	0.047	....	....	....	....	....	....	....	....	....	....	....	....
23									....	....	....	....	....	....	....	....	....	....	....	....	0.004	0.191	0.305	0.020	0.020	0.035
24	Aug. 8	1	680	680	June 3	66	400	59	....	....	....	....	....	....	....	....	.304	.086	.031	.062	....	....	....	....	....	....
25									....	....	....	....	....	....	....	....	....	....	....	....	.031	....	....	.039	.031	.039
26									....	....	....	....	....	....	0.055	.062	.601	.172	.094	.094	....	....	....	....	....	....
27	Aug. 9	1	368	368	June 3	67	204	55	....	....	....	....	....	....	....	....	....	....	....	....	.059	.086	.211	.078	.07[illegible]	.062
28									.062	....	0	.023	.055	.078	....	....	....	....	....	....	....	....	....	....	....	....
29									.105	.016	.035	.070	.070	.094	....	....	....	....	....	....	....	....	....	....	....	....
30	Aug. 11	5	365	73	July 12	30	170	47	....	....	....	....	....	....	.062	.094	....	.250	.094	.191	....	....	....	....	....	....
31									....	....	....	....	....	....	....	....	....	....	....	....	.082	.257	.398	....	.07[illegible]	.098
32									.039	.023	0	.031	.016	.055	....	....	....	....	....	....	....	....	....	....	....	....
33	Aug. 21	1	500	500	July 12	40	245	49	....	....	....	....	....	....	.020	.035	.222	.078	.016	.039	....	....	....	....	....	....
34									....	....	....	....	....	....	....	....	....	....	....	....	.020	.047	.117	0	.031	.035
35									.059	....	0	.047	.031	.051	....	....	....	....	....	....	....	....	....	....	....	....
36	Aug. 29	1	350	350	June 2	88	180	51	....	....	....	....	....	....	.062	....	.296	.070	.055	.094	....	....	....	....	....	....
37									....	....	....	....	....	....	....	....	....	....	....	....	.051	.09[illegible]	.152	.070	.07[illegible]	.062
38									.094	.039	.039	.090	.086	.121	....	....	....	....	....	....	....	....	....	....	....	....
39	Sept. 8	1	375	375	June 2	98	235	63	....	....	....	....	....	....	.039	.101	.425	.187	.09[illegible]	.062	....	....	....	....	....	....
40									....	....	....	....	....	....	....	....	....	....	....	....	.0[illegible]2	.094	.242	.062	.101	.062

[a] Leuco base of malachite green.

the curve generally begins. The juice from the plant collected on the sixty-seventh day seems unusually rich in oxidases. If the points obtained from the data on this plant were discarded, the curves would be all quite regular, with the exception of those corresponding to the oxidation of hydrochinone and of some of the cresols. To show what types of

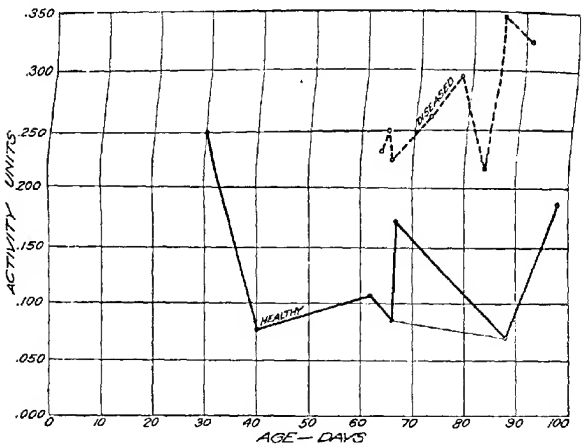


FIG. 8.—Curve showing oxidation of phloridzin in the presence of the juice of potato foliage.

curves would be obtained by elimination of the points obtained for the sixty-seventh day of growth, which point seems irregular, the adjacent points on both sides of the "sixty-seventh-day point" are connected with relatively thin lines to complete the curves; the initial fall and the final rise thus become very apparent and clear-cut.

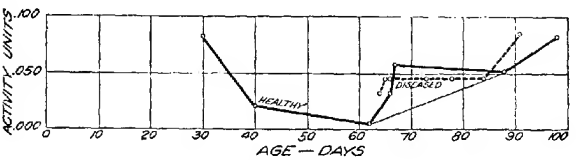


FIG. 9.—Curve showing oxidation of o-cresol in the presence of the juice of potato foliage.

The curves, of course, are not smooth. This is to be expected when it is considered that there are numerous factors influencing the physiological condition of the plants. Differences in the nature of the seed, of the soil, and many other factors probably influence the development of the plant qualitatively as well as quantitatively.

With all of the reagents except guaiacol and metacresol, the rise in the oxidase content observed during the second half of the period of examination coincides approximately with the stoppage of growth, which point is shown in figure 1 to be about the sixty-seventh day. In this respect, therefore, these results are in striking harmony with those obtained while working on diseased sugar beets. In the case of sugar beets the writer has shown that the factors which had a retarding influence on the growth of the plants also caused the oxidase content of the juice of their foliage

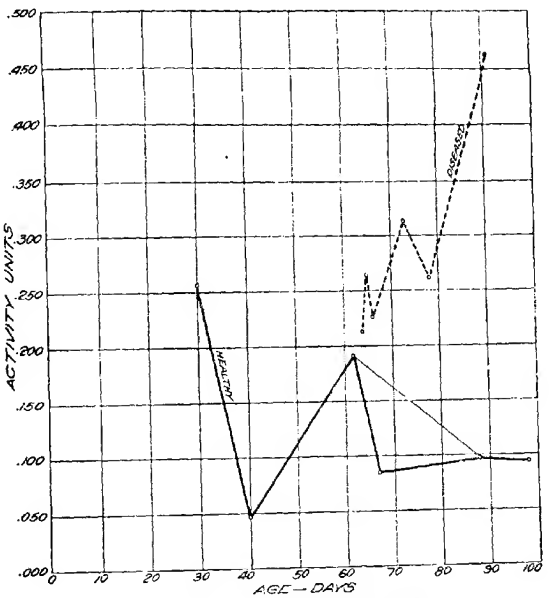


FIG. 10.—Curve showing oxidation of m-cresol in the presence of the juice of potato foliage.

to increase. It is possible that the same factors which led to an increased oxidase content during the retardation of growth of sugar beets will lead to a similar rise during normal cessation of growth in potato plants.

OXIDASE ACTIVITY OF THE JUICE OF THE SPROUTS AND OF THE TUBERS FROM WHICH THE SPROUTS HAD BEEN REMOVED

The relatively high oxidase content of very young potato plants suggested an examination of the sprouts from the tubers. Seed tubers of the Green Mountain variety of the same stock as was used for all of the

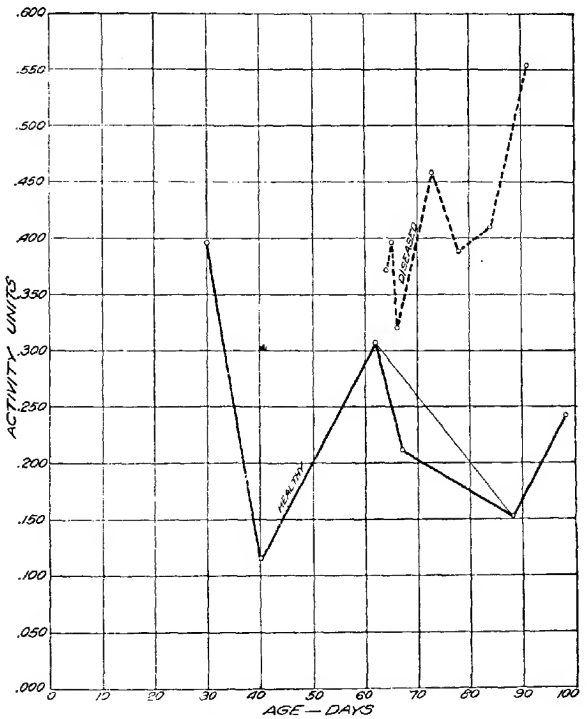


FIG. 11.- Curve showing oxidation of p-cresol in the presence of the juice of potato foliage.

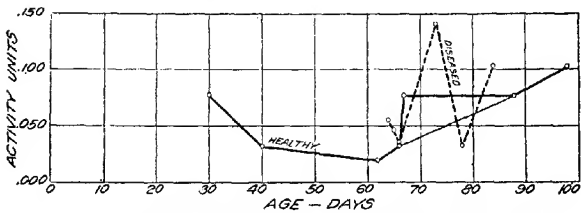


FIG. 12. -Curve showing oxidation of m-toluidin in the presence of the juice of potato foliage.

experiments already described in this paper were stored at room temperature from July 12 to September 3, 1913. During that time the tubers were lying on a table exposed to the light, and the temperature during the period fluctuated between 15° and 25° C. There were 36 tubers, soft but sound, yielding 170 grams of sprouts; the 36 tubers from which the sprouts had been removed weighed 2,670 grams. Only 22 c. c. of juice were obtained from the sprouts in the usual way. This juice turned immediately to a chocolate-brown color. The oxidase activity of the juice of the sprouts toward seven of the reagents is given in Table V. The oxidase activity of the juice of the tubers toward all of the reagents is given in Table VI.

TABLE V.—*Oxidase activities of the juice of the sprouts from Green Mountain seed-potato tubers*

Series No.	Oxidase activity expressed in units as measured in the oxidation of the reagents.						
	Pyrocatechol.	Tyrosin.	Phloridzin.	Resorcin.	Hydrochinone.	Guaiacol.	Paracresol.
41	0. 562	0. 296	0. 359	0. 055	.....	.....	.....
42	.....	.....	.....	.....	0. 807	0. 488	0. 495

[1] Leuco base of malachite green.

OXIDASE ACTIVITY OF THE JUICE OF THE TUBERS

The method of procedure in the case of the potato tubers was the same as that used with the foliage. The tubers were freed from adhering soil by means of cold, running water and were wiped dry with a clean towel and ground up whole. It has been known for some time that the juice obtained from the layers of the potato tuber near the surface is more active than that from the inner portions. Notwithstanding this variation of oxidase activity in different parts of the tuber, the whole tubers, including the peel, were used for these experiments. This was done to avoid the introduction of new factors. The results are presented in Table VII.

To see whether the oxidase content of the juice from the tubers bears any relation to either the age of the plant from which the tubers are derived or their own weight, the ratio of oxidase content to age and weight was represented graphically. In the figures 13 to 20 (see footnote, p. 379) two sets of curves are given. In both sets the oxidase activities of the juices are shown on the ordinates, while the ages and weights, respectively, are laid off on the abscissæ. The curves formed by the continuous lines show the relation of age to oxidase content and the curves formed by the thin broken lines show the relation of the size of the tubers to their oxidase content.

From these curves no definite relationship is apparent between the oxidase content of the tubers on the one hand and their age or size on

TABLE VI.—*Oxidase activities of the juice of healthy potato tubers which sprouted in the laboratory and from which the sprouts had been removed*

Series No.	Oxidase activity expressed in units as measured in the oxidation of the reagents.																				
	Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	Orthocresol.	Metacresol.	Paracresol.	Orthotoluidin.	Metatoluidin.	Paratoluidin.			
43	0.332	0.201	0.140	0.012	0.047	0.113	0.413	0.230	0.710	0.273	0.251	0.582									
44													0	0.257	0.952	0	0.012	0.150			
45																					

[a] Leuco base of malachite green.

TABLE VII.—*Oxidase activities of the juice of healthy potato tubers*

Series No.	Series No. of leaves on same plant.	Date of collection.	Date of experiment.	Number of hills used.	Total number of sprouts.	Total number of tubers.	Number of tubers per hill.	Number of tubers per sprout.	Total weight of tubers.	Average weight of tubers.	Age of plant.	Oxidase activity expressed in units as measured in the oxidation of the reagents.																	
												Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	O-cresol.	M-cresol.	P-cresol.	O-toluidin.	M-toluidin.	P-toluidin.
									Grams.	*Grams.*	*Days.*																		
46												0.121	0.137	0.070		0	0													
47	21	Aug. 4	Aug. 13	2	2	13	6.5	3.2	114	8.8	62							0.281	0.082		0.257	0	0.218	0	0.371	0.515	0	0	0.043
48																													
49												.205	.218	.117	(?) 0.016	.087	.023		.167	.140	.062	.281	.047	.432					
50	32	Aug. 21	Aug. 23	5	32	191	6.2	3.2	364	5.6	90													.955		.507	0	.023	.004
51													.263	.031		.023	.027	.0											
52																													
53	35	Aug. 29	Aug. 30	1	1	5	5	5	470	94.0	88							.170	0		.476	.231	.008	.062					
54																								.010		.655	0	.008	.008
55												.170	.191	.172		.008	.016	.047											
56	38	Sept. 8	Sept. 9	1	1	4	4	4	240	60.0	98							.220	.086	.380	.242	.008	.231	0	.406	.931	0	0	0
57																													

[a] Leuco base of malachite green.

the other. The irregularities are due, no doubt, to differences between individual samples and to slight differences in the mode of preparation of the juice. Great care was used to maintain uniformity of technique throughout this work, so that it does not seem very likely that the latter factor plays a rôle in the variations of oxidase contents observed.

OXIDASE ACTIVITY OF THE JUICE OF SEED TUBERS

In connection with these results the oxidase activity of the seed tubers of the plants used in these experiments seemed of interest. The average weight of the tubers when examined was 90 grams. The oxidase determinations were started on July 18, when the tubers had just begun to sprout. The results are given in Table VIII.

TABLE VIII.—*Oxidase activities of the juice of seed potato tubers from which all the healthy material used in this investigation was obtained*

Oxidase activity expressed in units as measured in the oxidation of the reagents.

Series No.	Date.	Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin	Alcoh.	Pyrocatechol.	Tyrosin.	Hydrochinone
58	July 18						0.037	0.261	0.129	0.540
59	July 18	0.179								
60	July 19									
61	July 19						.055			
62	July 19	.220				0.227				
63	July 24									
64	July 24		0.234	0.125	0.269					

Oxidase activity expressed in units as measured in the oxidation of the reagents.

Series No.	Phloridzin.	Resorcin	Guaiacol.	Orthocresol.	Metacresol.	Paracresol.	Orthotoluidin.	Metatoluidin.	Paratoluidin.
58									
59			0.312				0.008	0.030	0.094
60				0.047	0.339	0.913			
61									
62			.257						
63	0.359	0.008							
64									

[a] Leuco base of malachite green.

OXIDASES OF CURLY-DWARF-DISEASED POTATO PLANTS

The plot of potatoes from which all the normal material was collected turned out to be remarkably free from curly dwarf. The pathological material was therefore collected on a larger field several miles remote from the other. About 10,000 different kinds of potatoes were grown in the field, and on account of the comparative scarcity of the pathological material samples were chosen from a number of different varieties. In all of the experiments the name of the variety is stated whenever it is known. The conditions of growth with reference to soil and atmospheric conditions were practically the same in the case of both the diseased and healthy potatoes.

TABLE IX.—*Oxidase activities of the juice of shoots of curly-dwarf potato plants*

Series No.	Date.	Number of variety.	Name of variety.	Number of plants.	Total weight of shoots.	Mean total weight of shoots per hill.	Date of planting.	Age of plant.	Oxidase activity expressed in units as measured in the oxidation of the reagents.																				
									Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	O-cresol.	M-cresol.	P-cresol.	O-toluidin.	M-toluidin.	P-toluidin.				
					Gms.	*Gms.*		*Days.*																						
65	July 17	Unknown.	Unknown	10	361	36	June 2	45	....	0.043	....	....	....	0.039	0.078	0.030	....	....	....	....	....	....	....	....	....	....	....	....	....	....
66									....	.031	....	....	....	.062	....	....	.066	0.359	....	....	0.125	....	....	....	....	....	....	....	....	....
67	July 21	do	do	10	435	44	do	49	....	....	....	....	....	....	....	....	....	.266	....	....	.094	....	....	....	....	....	....	....	....	....
68									....	....	....	....	....	....	....	....	....	....	....	....	....	0.023	0.312	0.445	....	....	....	....	....	....
69	July 22	do	do	7	260	37	do	50	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	0.047	0.057	0.117	....
70	July 23	do	do	7	260	37	do	51	....	....	....	....	0.031	....	....	....	....	0.220	0.047	....	....	....	....	....	....	....	....	....	....	....
71									0.074	....	0.023	0.047	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
72									.062	....	....	....	.020	....	.031	.047	....	....	.063	....	....	....	....	....	....	....	....	....	....	....
73	July 28	do	do	3	111	37	do	56	....	....	0	....	.016	....	.020	....	....	.374	.187	....	.078	....	....	....	....	....	....	....	....	....
74									....	....	....	....	....	....	....	....	....	....	....	....	....	.039	.140	.382	0	0	....	.012	....	....
75	July 29	do	do	4	370	93	do	57	....	....	....	....	....	....	.078	.023	.432	.371	.031	.078	....	....	....	....	....	....	....	....	....	....
76									.062	....	....	.055	.016	.023	.023	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
77		6652	Daisy×Round Pinkeye.						.140	.086	.031	.094	.062	.094	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
78	July 30	4046	Prof. Maerker ×Silverskin.	5	150	30	do	58	....	....	....	....	....	....	.109	.078	.659	.304	.047	.140	....	....	....	....	....	....	....	....	....	....

[a] Leuco base of malachite green.

OXIDASE ACTIVITY OF THE JUICE OF THE SHOOTS

The procedure was very much like that used with healthy potato plants. The first experiments were carried out on the whole shoot of the plants. The results are summarized in Table IX.

There is no definite tendency observable in these results. The diluting influence of the stems is apparently more than compensated for by the

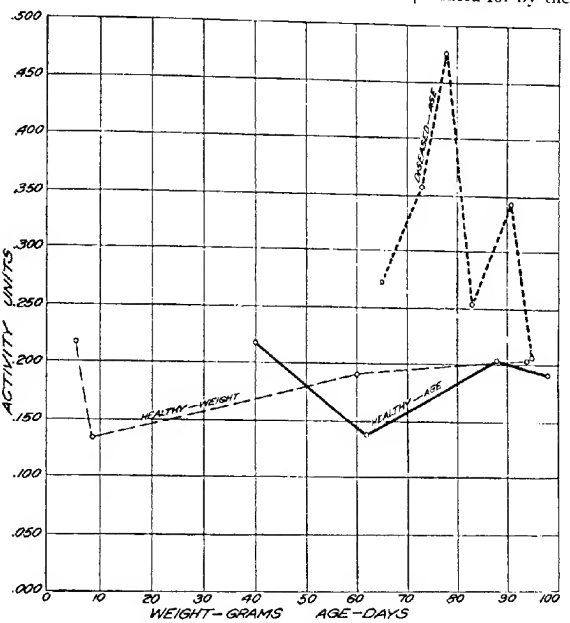


FIG. 13. Curve showing oxidation of pyrogallol in the presence of the juice of potato tubers.

relatively higher oxidase content of the foliage of the older plants. All of the plants were started at the same time, so that from the viewpoint of age the results are comparable. As is shown by Table IX, no direct correlation can be found between the age or weight of the shoot and the oxidase content. These results are further discussed, together with other data on diseased foliage, on page 399.

OXIDASE ACTIVITY OF THE JUICE OF THE LEAVES

The results obtained in working with the foliage of curly-dwarf potato plants rather than with the whole shoots are given in Table X.

TABLE X.—*Oxidase activities of the juice of the leaves of curly-dwarf potato plants*

Series No.	Date.	No. of variety.	Number of hills used.	Total weight of shoots.	Mean total weight of shoots per hill.	Number of shoots.	Mean weight of shoots.	Date of planting.	Age of plant.	Total weight of stems.	Stems × 100 ÷ total weight of shoots.	Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	O-cresol.	M-cresol.	P-cresol.	O-toluidin.	M-toluidin.	P-toluidin.
				Gms.	*Gms.*		*Gms.*		*Days.*	*Gms.*	*Gms.*	Oxidase activity expressed in units as measured in the oxidation of the reagents.																	
79																		0.070	0.059		0.234	0.062	0.078						
80	Aug. 5	[b]14980	4	380	95	4	95	June 2	64	178	47	0.062	0.039	0	0.047	0.055	0.070												
81																								0.031	0.211	0.371	0.031	0.055	0.078
82												.109		.047	.086														
83	Aug. 6	[c]5014, [d]5438	3	440	147	12	37	...do...	63	234	58							.078	.062		.250	.039	.055						
84																								.047	.265	.398	.020	.047	
85													.062		.016	.027	.062			0.374									.062
86																		.016	.055	.367	.226	.027	.070						
87	Aug. 7	.....	3	530	177	14	38	...do...	66	284	54	.031	.035	0	.031	.008	.035												
88																								.047	.226	.320	.031	.035	.023
89												.101	.043	.016	.055	.051	.059												
90	Aug. 14	[e]2203	4	642	161	15	43	...do...	73	315	49							.117	.047	.585	.261	.055	.137						
91																								.047	.312	.460	.078	.140	.055
92												.039	.016	0	.039	.016	.031												
93	Aug. 19	.....	4	500	125	20	25	...do...	78	198	40							.101	.043	.406	.296	0	.105						
94																								.047	.261	.390	.043	.031	.055
95												.070		0	.062	.051	.078												
96	Aug. 25	[f]6061	2	890	445	5	178	...do...	84	440	50							.078	.035	.515	.218	.055	.086						
97																								.047		.410	.062	.101	.078
98	Aug. 27	[g]23457	5	550	110	7	79	...do...	86	210	38							.078	.098		.347		.125						
99												.016 (?)	.047	.113	.121	.105													
100	Sept. 1	[h]18743	4	440	110	7	6[illegible]	...do...	91	180	4[illegible]							.148	.117		.324	.086	.156						
101																								.086	.464	.554			

[a] Leuco base of malachite green.
[b] Keeper × Silverskin.
[c] Pres. Kruger × Keeper.
[d] Prof. Maerker × Apollo.
[e] Sophie × Keeper.
[f] Daisy × Keeper.
[g] Early Eureka × Keeper.
[h] S. demissum × Keeper.

In order to ascertain whether the oxidase content of the leaves of these abnormal plants showed with age similar fluctuations to those found in healthy plants, curves representing the measurements of these oxidases were plotted with the curves representing the oxidase measure-

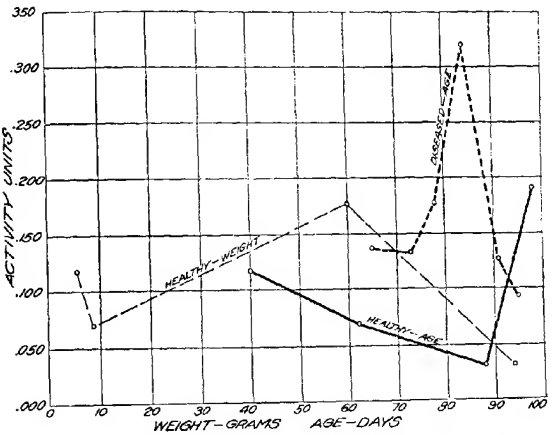


FIG. 14.—Curve showing oxidation of α-naphthol in the presence of the juice of potato tubers.

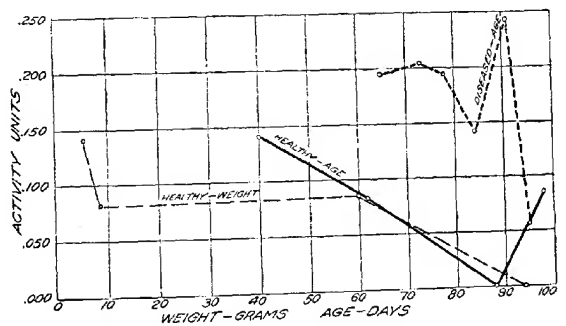


FIG. 15.—Curve showing oxidation of tyrosin in the presence of the juice of potato tubers.

ments of the healthy plants. Some of these are shown by the broken lines in figures 6 to 12. Plotting both curves on the same system of coordinates has the additional advantage of making possible a quick observation of the comparative magnitudes of oxidase activity in the healthy and diseased juices.

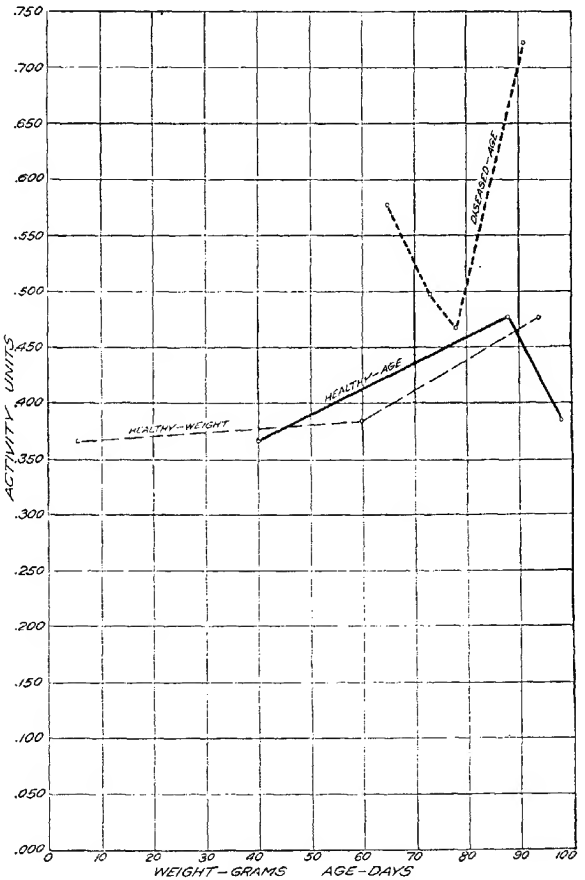


FIG. 16.—Curve showing oxidation of hydrochinone in the presence of the juice of potato tubers.

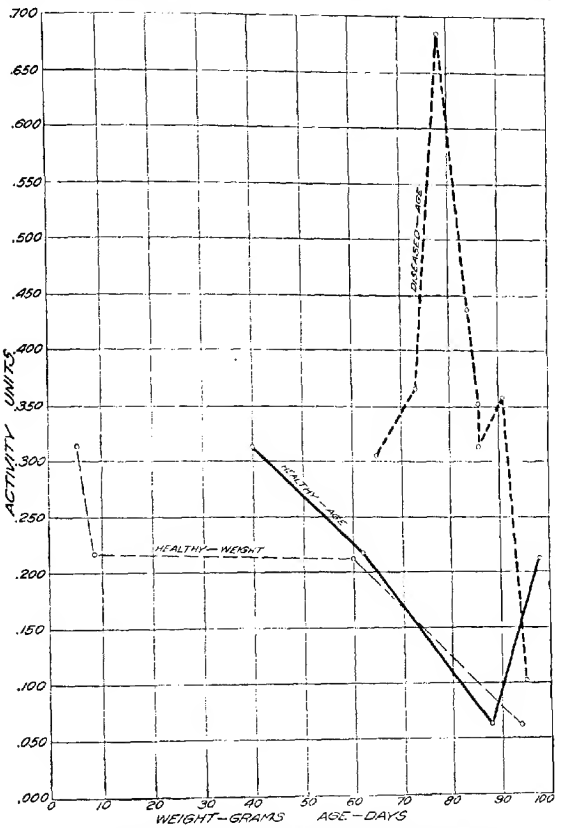


FIG. 17.—Curve showing oxidation of guaiacol in the presence of the juice of potato tubers.

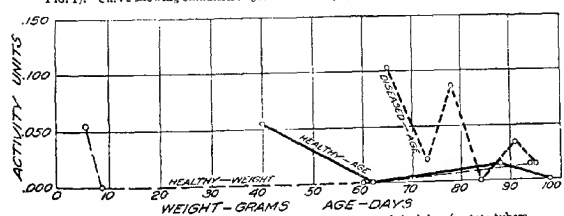


FIG. 18.—Curve showing oxidation of o-cresol in the presence of the juice of potato tubers.

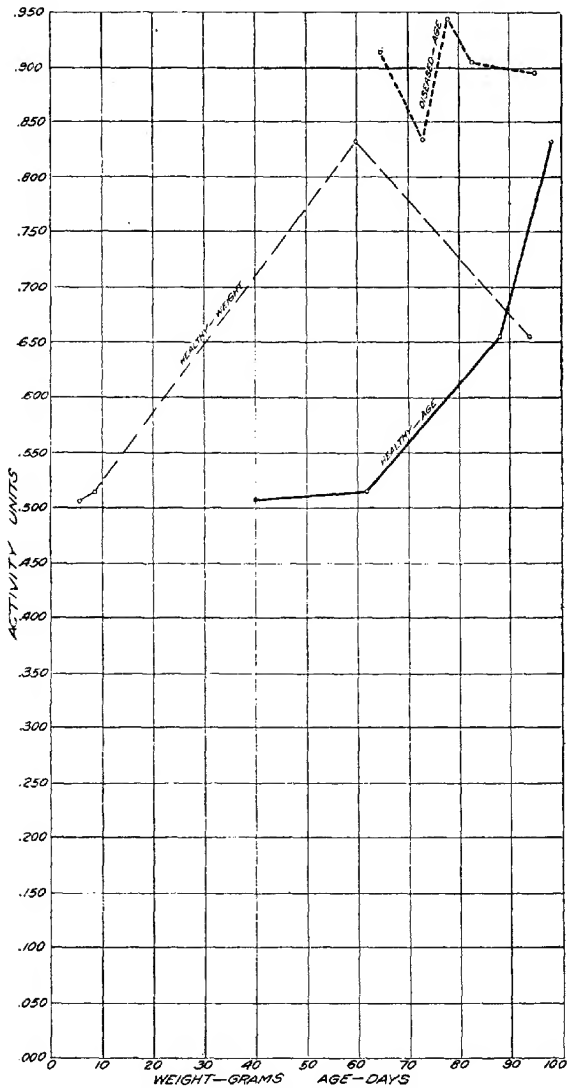


FIG. 19.—Curve showing oxidation of p-cresol in the presence of the juice of potato tubers.

These curves seem to show no definite tendency such as was seen in the case of the growing leaves of healthy plants. This is to be expected when it is considered that the physiological condition of the plants and presumably the oxidase contents of the juices are here influenced by two factors combined, age and disease. Past experience has shown that the oxidase activity of the plant juices is markedly affected by physiological disturbances such as the curly-dwarf disease of potatoes seems to be. The magnitude of the effect on the oxidase activities probably depends on such factors as the age of the plant when the diesase first took hold,

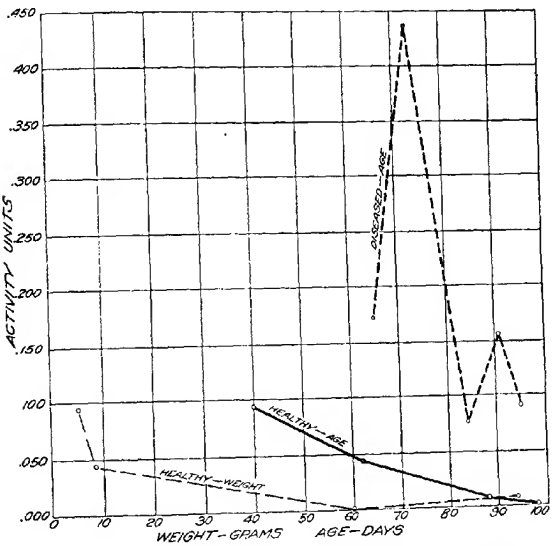


FIG. 20.—Curve showing oxidation of p-toluidin in the presence of the juice of potato tubers.

the length of time elapsed since, the individual resistance of the plant, etc. None of the factors are susceptible of measurement; the plants examined are influenced by them to different degrees and are therefore not comparable.

However, they all showed the typical curly-dwarf symptoms, and if the oxidase activities of the leaf juice are influenced by the apparent physiological disturbances the influence should be noticeable by a deviation of the oxidase activities from the normal and in a definite direction. That such a deviation from the normal actually exists is indicated by the curves in figures 6 to 12. With most of the reagents used the broken

lines, representing the oxidase activities of the curly-dwarf foliage, run at a higher mean level than the continuous lines, which represent the oxidase activities of the healthy foliage. The differences will be brought out in a mathematical form in a latter part of this paper.

To get a clear idea of the striking differences in the rate and extent of growth existing between the healthy and the diseased plants, the results showing these differences are represented graphically in figure 21. The ages of the plants are represented on the abscissæ, the mean weight

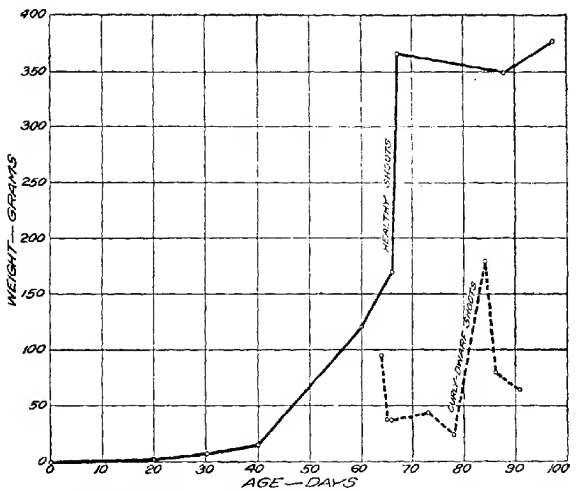


FIG. 21.—Curve showing the differences in rate and extent of growth between healthy and diseased plants.

of the shoots on the ordinates. The continuous line represents the growth of the healthy plants, the broken line that of the diseased ones.

The difference in weight of the two types of potato plants of the same age is strikingly apparent. The diseased plants made an average growth of only about one-eighth of the growth of the normal plants.

The fluctuations in the curve representing the rate of growth of curly-dwarf shoots are to be expected when the complex nature of the disease is considered. The diseased plants used for experimentation, although showing the typical symptoms, differed greatly in size, as is shown in figure 21, in color, and also in the shape which they assumed on account of the inhibition of growth.

OXIDASE ACTIVITY OF THE JUICE OF THE TUBERS

The collection of the tubers of the diseased plants and the determinations of the oxidase content of the juices obtained from them were carried out in the same way as was done in the case of the healthy material. The results are summarized in Table XI and are included in figures 13 to 20. As before, the heavy broken lines represent the results obtained with curly-dwarf material.

These results, like those obtained with the tubers of healthy potato plants, show no definite tendency. If with age there is a definite variation in oxidative capacity exhibited toward all of the reagents, it is entirely masked by the irregular fluctuations. These irregular fluctuations were also observed in the case of diseased foliage and are illustrated in figures 6 to 12.

DISCUSSION OF RESULTS

Comparison of the curves of the healthy plants with those of the diseased ones shows at a glance a greater oxidase activity in the case of the curly-dwarf material. This is true for both the tubers and the foliage. It seemed desirable to express these differences in some numerical form, and this was done by taking the averages of all the results obtained from material of the same type with the same reagents. These averages were then easily compared.

It was shown that healthy foliage yields juices of diminishing oxidase activity from the time of sprouting up to about the fortieth day of growth (as counted from the time of planting). For this reason in this summary of averages must be included only those of the results obtained with healthy leaves which were obtained during the growth periods of the diseased material examined. The age of the diseased foliage collected ranged from 64 to 91 days; the age of the plants where the whole shoots were examined was from 45 to 58 days. The averages were calculated as follows: All of the data (oxidase activities) obtained within the age periods mentioned were added together with the figures obtained for the beginning and the end of the period by interpolation from the curve. The sum, of course, was divided by the number of data added. These averages are shown in Table XII.

TABLE XI.—*Oxidase activities of the juice of potato tubers*

Series No.	Series No. of leaves of same.	Date of collection.	Date of experiment.	Number of hills used.	Total number of shoots in same.	Total number of tubers.	Number of tubers per hill.	Number of tubers per shoot.	Total weight of tubers.	Mean weight of tubers.	Age of plant.	Oxidase activity of juice of tubers expressed in units using the reagents.																	
												Benzidin.	Pyrogallol.	α-naphthol.	L. b. of m. g.[a]	Phloroglucin.	Aloin.	Pyrocatechol.	Tyrosin.	Hydrochinone.	Phloridzin.	Resorcin.	Guaiacol.	O-cresol.	M-cresol.	P-cresol.	O-toluidin.	M-toluidin.	P-toluidin.
									Gms.	*Gms.*	*Days.*																		
102												0.413	0.273	0.137	0.059	0.035	0.047	...	...	...	...	...	...	...	...	...	...	...	...
103	82	Aug. 6	Aug. 12	3	12	11	3.7	0.9	245	22.3	65	...	...	...	...	...	...	0.425	0.195	0.577	0.211	0	0.304	...	...	...	...	...	...
104												...	...	...	...	...	...	...	...	...	...	...	...	0.101	0.647	0.913	0.004	0.031	0.172
105												...	.359	.133	.031	.023	.023	...	...	...	...	...	...	...	...	...	...	...	...
106	89	Aug. 14	Aug. 15	4	15	27	6.8	1.8	175	6.5	73	...	...	...	...	...	...	.082	.203	.499	.234	.008	.367	...	...	...	...	...	...
107												...	...	...	...	...	...	...	...	...	...	...	...	.020	.515	.835	0	.031	...
108												...	.468	.179	.023	...	.070	...	...	...	...	...	...	...	...	...	...	...	...
109	92	Aug. 19	Aug. 20	4	20	94	23.5	4.7	365	3.9	78	...	...	...	...	...	...	.472	.195	.408	.257	...	.686	...	...	...	...	...	...
110												...	...	...	...	...	...	...	...	...	...	...	...	.086	.593	.944	.008	...	.437
111												.250	.254	.320	0	.031	0	...	...	...	...	...	...	...	...	...	...	...	...
112	95	Aug. 25	Aug. 26	2	5	15	7.5	3.0	530	35.3	84	...	...	...	...	...	...	.281	.140	...	.289	0	.437	...	...	...	...	...	...
113												...	...	...	...	...	...	...	...	...	...	...	...	0	.218	.905	0	0	.078
114	98	Aug. 27	Aug. 28	5	7	14	2.8	2.0	505	36.0	86	...	...	...	...	...	...	...	...	...	.265	...	.312	...	...	...	...	...	...
115												...	...	...	...	...	...	...	...	...	.234	0	.351	...	...	...	...	...	...
116												.086	.343	.129	0	.023	.008	...	...	...	...	...	...	...	...	...	...	...	...
117	99	Sept. 1	Sept. 2	4	7	17	4.3	2.4	265	15.6	91	...	...	...	...	...	...	.078	.242	.722	.254	0	.359	...	...	...	...	...	...
118												...	...	...	...	...	...	...	...	...	...	...	...	.035	.460	...	0	0	.156
119												.176	.207	.094	.016	.031	.043	...	...	...	...	...	...	...	...	...	...	...	...
120	(b)	Sept. 5	Sept. 6	3	...	33	11	...	590	17.9	95	...	...	...	...	...	...	...	.059	...	.195	.016	.101	...	...	...	...	...	...
121												...	...	...	...	...	...	...	...	...	...	...	...	.016	...	.897	0	0	.090

[a] Leuco base of malachite green.

[b] Foliage dead.

TABLE XII.—*Relative oxidase activity of healthy and curly-dwarf-diseased potatoes*

Reagent used.	Oxidase activity[a] of juices of—						Difference in activity between juices of—		
	Shoots of healthy plants collected at the age of 45 to 58 days.	Shoots of curly-dwarf plants collected at the age of 45 to 58 days.	Foliage of healthy plants collected at the age of 64 to 91 days.	Foliage of curly-dwarf plants collected at the age of 64 to 91 days.	Tubers of healthy plants.	Tubers of curly-dwarf plants.	Curly-dwarf and healthy shoots.	Curly-dwarf and healthy foliage.	Curly-dwarf and healthy tubers.
							Per cent.	*Per cent.*	*Per cent.*
Benzidin	0.011	0.085	0.062	0.061	0.188	0.231	+ 673	− 2	+ 23
Pyrogallol	.002	.053	.026	.040	.187	.318	+2,550	+ 54	+ 70
α-naphthol	.011	.027	.008	.025	.098	.165	+ 145	+213	+ 68
Leuco base of malachite green	.014	.043	.040	.057	.015	.022	+ 207	+ 42	+ 47
Phloroglucin	.017	.034	.045	.045	.023	.029	+ 100	0	+ 26
Aloin	.013	.048	.065	.056	.023	.032	+ 269	− 14	+ 39
Pyrocatechol	.027	.076	.066	.086	.261	.268	+ 184	+ 54	+ 3
Tyrosin	.016	.057	.068	.064	.027	.132	+ 256	− 6	+123
Hydrochinone	.312	.450	.382	.450	.469	.567	+ 44	+ 18	+ 39
Phloridzin	.112	.272	.106	.269	.248	.243	+ 143	+154	− 2
Resorcin	.023	.042	.058	.040	.016	.004	+ 83	− 21	− 75
Guaiacol	.047	.103	.079	.102	.200	.364	+ 119	+ 29	+ 82
O-cresol	.041	.031	.045	.059	.018	.043	− 24	+ 11	+139
M-cresol	.169	.226	.108	.290	.388	.487	+ 34	+168	+ 25
P-cresol	.266	.414	.203	.414	.627	.919	+ 56	+103	+ 47
O-toluidin	.036	.024	.057	.044	0	.002	− 33	− 23	. . . .
M-toluidin	.040	.029	.060	.068	.008	.012	− 28	+ 13	+ 50
P-toluidin	.028	.065	.052	.039	.036	.187	+ 132	− 25	+419

[a] Activity expressed in units as measured in the oxidation of the reagents.

As Table XII shows, the differences existing between the oxidase activity of the healthy and of the diseased material are generally marked and the greater activity is in the curly-dwarf potato plants. The comparison of the data for healthy and curly-dwarf shoots shows that among the 18 reagents only 3 are oxidized more readily in the presence of the juice of the healthy plants. Comparison of the leaves of the two types of plants shows 7 of the 18 reagents to be more readily oxidized by the healthy juice; in the case of the two types of tubers only two of the reagents showed greater oxidation by the healthy material. Among 54 sets compared, 12 showed a greater activity in the case of the healthy material, while the remainder, 42, showed a much greater activity in the case of the diseased plants.

It seems safe to conclude that in general the oxidizing power in the juices of curly-dwarf potato plants is greater than in those of healthy plants. The writer does not know as yet exactly what bearing, if any, the oxidases measured by him have on the oxidation processes going on in the cells. A priori, one would conclude that the intensity of oxidation processes in the cells would among other factors depend on the concentration of the various oxidases present. If this were the case, one would expect cell respiration to be more intense in the cells of the curly-dwarf tubers. The diseased plants would be in a condition corresponding to "fever" in animals.

These results agree in their general nature with those obtained in the case of the curly-top of sugar beets (Bunzel, 1913a, 1913b) and the leaf-roll of potatoes (Doby, 1911–12). In all three cases an increase in oxidases and a general retardation of growth are found. It would be extremely interesting, especially to plant physiologists, to find out what the rate of respiration is in such dwarfed, presumably "feverish" plants. Experiments intended to throw light on this point are already being planned in the laboratory of the Office of Plant Physiological and Fermentation Investigations.

There are a number of facts brought out in this investigation which open doors to new aspects of the physiology of development. There seems to be a cycle in the activity of the expressed juice of the foliage of normally developing potato plants. The juice of the foliage of very young plants is more active than that of plants of the same variety 40 or 50 days older; after that stage of development the activity rises again with increasing age. Quite in harmony with these findings is the fact that sprouts of artificially sprouted tubers of the same variety are much more active than the youngest foliage examined.

There seems to be a parallelism, therefore, between the intensity of physiological activity and the quantity of oxidases present. This belief is strongly corroborated by the fact that the physiologically more active portions of the plant, such as the leaves, furnish juices with greater activity than the obviously less active portions of the same plant, such as

the stems. This has been found by the writer not only in the case of the potato plants, but also in sugar beets (Bunzel, 1913a, 1913b).

In this connection the results obtained by Nicolas (1907) are very interesting. He studied the respiration of individual parts of plants and found that those organs which carry out the assimilating functions of the plant show the greatest respiratory activity. The limbs or the organs which replace them in function, such as the phyllodia or cladodia, have 1.4 to 4.5 times as great a respiratory activity as the petiole, stem, or tendrils. These results when combined with those obtained by the writer in the present investigation would indicate that there is at least a general parallelism between the oxidase activity of the juice obtained from a plant organ and the intensity of its physiological activity, as measured by its intensity of respiration. Plans are made to study the question more closely in the laboratory of the Office of Plant Physiological and Fermentation Investigations.

SUMMARY

(1) The oxidase activity of the foliage of normally developing potato plants is greatest in the early stages of development; it falls off with growth of the plants and rises again when the plant's growth about reaches a standstill.

(2) Curly-dwarf potato plants show a greater oxidase activity than healthy ones of the same age, both in the juice of their tubers and in the juice of their foliage.

(3) The oxidative activity of the different parts of the potato plant has been established for 18 different reagents.

LITERATURE CITED

BACH, A., and CHODAT, R.
1904. Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. *In* Ber. Deut. Chem. Gesell., Jahrg. 37, no. 10, p. 2434–2440, 2 fig.

BERTRAND, GABRIEL.
1897. Sur l'intervention du manganèse dans les oxydations provoquées par la laccase. *In* Compt. Rend. Acad. Sci. [Paris], t. 124, no. 19, p. 1032–1035.

BUNZEL, H. H.
1912. The measurement of the oxidase content of plant juices. U. S. Dept. Agr. Bur. Plant Indus. Bul. 238, 40 p., 9 fig., 2 pl.
1913a. A biochemical study of the curly-top of sugar beets. U. S. Dept. Agr. Bur. Plant Indus. Bul. 277, 28 p.
1913b. Die Rolle der Oxydasen in der Blattrollkrankheit der Zuckerrübe. *In* Biochem. Ztschr., Bd. 50. Heft 3/4, p. 185–208.

DIXON, H. H., and ATKINS, W. R. G.
1913. Osmotic pressures in plants. I. Methods of extracting sap from plant organs. *In* Sci. Proc. Roy. Dublin Soc., n. s. v. 13, no. 28, p. 422–432.

DOBY, GÉZA.
1911–12. Biochemische Untersuchungen über die Blattrollkrankheit der Kartoffel. *In* Ztschr. Pflanzenkrank., Bd. 21, Heft 1/2, p. 10–17, Heft 6, p. 321–336, 1911; Bd. 22, Heft 4, p. 204–211, Heft 7, p. 401–403, 1912.

DONY-HÉNAULT, OCT.

1908. Contribution à l'étude méthodique des oxydases. *In* Acad. Roy. Belg. Bul. Cl. Sci., 1908, no. 2, p. 105–107.

—— and VAN DUUREN, J.

1907. Contribution à l'étude méthodique des oxydases dans les tissus animaux. *In* Acad. Roy. Belg. Bul. Cl. Sci., 1907, no. 5, p. 537–638.

GRÜSS, J.

1907. Abhandlungen über Enzymwirkungen. *In* Ztschr. Pflanzenkrank., Bd. 17, Heft 2, p. 65–79, 1 fig., pl. 4.

KÖNIG, J., and KRÜSS, H.

1904. Erläuterungen zur Feststellung des Trübungsgrades und der Farbentiefe von Flüssigkeiten mittels des Diaphanometers. *In* Ztschr. Untersuch. Nahr. u. Genussmtl., Bd. 7, Heft 10, p. 587–590.

NICOLAS, G.

1907. Sur la respiration des organes végétatifs aériens des plantes vasculaires. *In* Compt. Rend. Acad. Sci. [Paris], t. 144, no. 20, p. 1128–1130.

ORTON, W. A.

1914. Potato wilt, leaf-roll, and related diseases. U. S. Dept. Agr. Bul. 64, 48 p., 16 pl. Bibliography, p. 44–48.

PALLADIN, W.

1906. Bildung der verschiedenen Atmungsenzyme in Abhängigkeit von dem Entwicklungsstadium der Pflanzen. *In* Ber. Deut. Bot. Gesell., Bd. 24, Heft 2, p. 97–107, pl. 8

SORAUER, PAUL.

1908. Die angebliche Kartoffelepidemie, genannt die "Blattrollkrankheit." *In* Internat. Phytopath. Dienst (Beigabe, Ztschr. Pflanzenkrank.), Jahrg. 1, Stück 2, p. 33–59.

TRILLAT, A.

1903. Influences activantes ou paralysantes agissant sur le manganèse envisagé comme ferment métallique. *In* Compt. Rend. Acad. Sci. [Paris], t. 137, no. 22, p. 922–924.

1904. Sur le rôle d'oxydases que peuvent jouer les sels manganeux en présence d'un colloïde. *In* Compt. Rend. Acad. Sci. [Paris], t. 138, no. 5, p. 274–277.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

25 CENTS PER COPY

SUBSCRIPTION PRICE PER YEAR, 12 NUMBERS, $2.50